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PULMONARY CEREBRAL EFFECT TIME AS DETERMINED BY AMYL NITRITE*

I. ESTABLISHMENT OF A NORMAL RANGE

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NUMEROUS methods have been devised for determining the rate of flow of blood from a given portion of the body to a distant designated locus. Since the work of Hering in 1827,[1] when he injected potassium ferrocyanide into one jugular vein of a horse, and tested samples of blood from the opposite jugular vein for Prussian blue, the greatest number of circulation time tests have involved the introduction of a chemical substance into a vein and the detection of that substance at some other point in the circulation by its objective or subjective manifestations.

Following in almost chronologic order is listed the evolution of circulation time studies: fluorescein[2]; calcium chloride[3]; concentrated salt solution producing distal electropotential changes[4]; radium C[5-7]; histamine flush reaction[8, 17]; phenoltetraiodophthalein sodium[9]; Congo red[10]; acetylcholine, determining volume changes in an extremity[11]; atropine and ephedrine, noting pupillary changes[12]; decholin[13-15, 21]; saccharine[16]; ether[18]; calcium gluconate[19]; magnesium sulfate, calcium gluconate, sodium chloride, copper sulfate mixture[20]; Kvale mixture,[22] and cyanide method.[23]

Other investigators have employed inhalation of various gases and have determined the rate of flow incidental to cardiac output studies, or the effect on the respiratory center; i.e., nitrous oxide method,[24] 5 to 7 per cent carbon dioxide inhalation,[25] rebreathing experiments with carbon dioxide,[26] ethyl iodide,[27, 28] and 50 per cent carbon dioxide inhalation.[29]

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All of the preceding, excepting the inhalation methods, necessitate single or double venipuncture. Those not relying upon subjective responses and their attendant variations, require in many instances complicated techniques or apparatus, making them almost prohibitive of widespread clinical application.

The method proposed in this report is readily controlled by a single operator, and obviates the use of venipuncture. It involves the inhalation of amyl nitrite, and the detection of drug action on the vessels of the head. The inhalation of amyl nitrite vapor is followed by its absorption into the pulmonary capillary bed, its transport to the left side of the heart, thence to the cerebral and peripheral vessels where objective and subjective phenomena may be noted. The arterial system of the head and neck is affected before the rest of the systemic circulation by virtue of the lesser distance to be traversed. Since the quantity of the drug is relatively small, and since the drug action is rapidly dissipated, peripheral systemic effects are seldom noted. A means is thus afforded for measuring the pulmonary cerebral effect time. Reasons for the use of this term will be made clear as the subject is developed in this report, which is limited to the establishment of a normal range of values for the method employed.

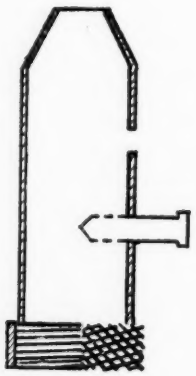

Fig. 1.—Inhaler.

APPARATUS AND TECHNIQUE

The amyl nitrite inhaler used for the test is a small cylindrical chamber (Fig. 1), 2 inches in length, with an inner diameter of ½ inch, and constructed of 1/16 inch aluminum tubing. It is fitted with a screw cap at one end, the opposite end tapering to an opening 5/16 inch in diameter. There are two ⅛ inch drill holes in the body. Into one of these is inserted a plunger with which to break the perles of amyl nitrite. A stop watch is the only other instrument necessary.

A 3 minim perle of amyl nitrite is placed in the chamber through the screw cap end, and the plunger is withdrawn. The operation is greatly facilitated by holding the chamber in the left hand, thumb at the screw cap end, middle finger over the opening at the tapered end, and index finger closing the first drill hole. The plunger is forced down with the right thumb, breaking the glass perle, its contents filling the chamber which is still sealed as described.

The patient is instructed that following a normal expiration, he is to inhale slowly and deeply from the inhaler (usually for 2 seconds) when it is brought to his nostril, and then to breathe normally again. The presence of abnormalities of the septum or turbinates is determined before this step, since an obstructed nasal passage would not permit inhalation of the drug. He is told that the medication inhaled will produce a "new feeling" or "new sensation" in his head, which will appear as a slight dizziness or fullness, followed by a feeling of warmth in the face and neck. The entire reaction will disappear within one to three minutes. The patient is asked to signal the observer immediately upon perception of any sensory change. The signal, previously agreed upon, consists either in raising the hand or saying "now." The starting time for the test is the time at which inhalation is completed, and the end point is the time indicated by the signal.

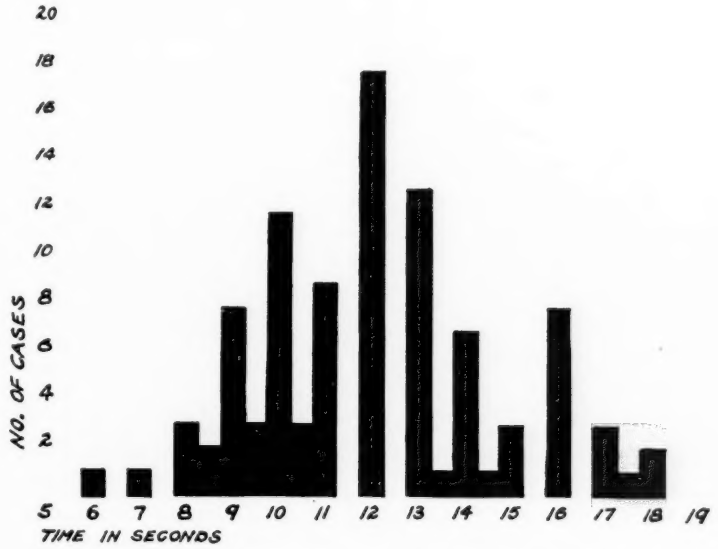

Fig. 2.—Pulmonary cerebral effect time in 100 cases.

RESULTS

One hundred persons selected at random from the interne, nursing, and house staff of Michael Reese Hospital were tested by the advocated method, and the results were noted. They are assembled in a graph form, shown in Fig. 2.

The greater majority of measurements lie between 10 and 16 seconds. The average time for the 100 cases is twelve seconds. This spread is no greater than that obtained by other methods currently in use.

In the proposed method for determining pulmonary cerebral effect time, the first manifestations are noted in the distribution of the external and internal carotid arteries. Occasionally, throbbing in the arms, and perspiration and warmth of the hands are later noted. A few persons have coughed after the full effect has been reached.

The only untoward reaction found in the series, from the use of amyl nitrite in the dose administered, was the presence of headache, persisting from ten minutes to three hours in three persons.

DISCUSSION

A number of factors must be given consideration in evaluating any circulation study. As a matter of fact, no result may be properly called "circulation time," unless objective and mechanically recorded intervals along the course of a given segment of the vascular tree being investigated are noted. Any instance in which a sensory response is taken as an end point lends itself to errors of latency in perception and expression. There is another phase of rate of flow studies that is not sufficiently emphasized. In those techniques involving passage of a test medium through the lung (e.g., inhalation and ether), there is an inherent and quite variable "across membrane" delay in that structure. Thus it is obvious that a more accurate term encompassing flow rate and end point manifestation is desirable. The term "effect time" is suggested for this purpose.

The possibility exists that amyl nitrite changes the circulatory tree through which it passes. The reaction of amyl nitrite has been investigated by Lauder Brunton in 1871, who noted its pronounced but transient arterial dilatation, attributing the phenomenon to a direct effect upon the smooth muscle of the blood vessels, independent of any nervous mechanism.[30] His investigations have been verified by innumerable subsequent investigators, who found that cerebral vessels,[31] pulmonary vessels,[32] coronary vessels,[33] splanchnics,[34] and peripheral vessels,[35] respond by dilatation. Pilcher and Sollman in 1915[36] showed that the vasomotor center is not influenced directly by the drug when they observed that there was no blood pressure fall or rise following introduction of amyl nitrite only into the cerebral circulation. Vagal depression occurs only as a compensatory response to the systemic fall in pressure, and is, therefore, not due to an isolated action of the drug. This was adequately demonstrated by Filehne,[37] who showed that when the pressure was kept level by partial clamping of the aorta, no increase in rate occurred.

The time interval between the inhalation and manifestation of drug action is dependent upon two main factors, aside from the reaction time, assuming that the dose is fairly uniform and the technique is constant: (1) circulatory dynamic factor; (2) respiratory dynamic factor.

The first of these factors, i.e., the circulatory dynamic factor, refers more specifically to those changes associated with the occurrence of cardiac failure and concomitant slowing of the circulation. The test method can be interpreted as an index of the circulation of the left side of the heart, since the drug enters the circulation at the lung capillaries and has its effect after passing through the blood vessels of the head. It does not measure rate of circulation through the right side of the heart.

However, the second factor, the respiratory dynamic factor, comes into play. Alterations in the pulmonary ventilation, total capacity, or alveolar transport, will affect the measurement. Thus chronic pulminary diseases, such as emphysema, bronchitis, bronchiectasis, and bronchial asthma, can affect the results noted.

The pulmonary cerebral effect time thus may be viewed as an additive phenomenon. Consequently, before a result is ascribed to an altered state of circula-

tion of the left side of the heart, the effects of pulmonary disease and abnormal reaction times must be considered in each case individually.

Furthermore, the circulation time itself is determined not only by the minute volume output of the heart, but also by the quantity of blood contained in the path from the lung capillaries to the head circuit. This is usually most variable in the pulmonary capillaries and veins, and next in the left auricle.

In short, amyl nitrite is a drug whose prime action is limited to a direct effect upon the vascular system, probably not associated with reflex dilatation or constriction. It may be used as a clinical test as suggested, even though the end point is a subjective one, necessitating recognition of the variations in individual reaction time. The discomfort associated with its use is relatively mild and disappears rapidly.

There is perhaps a single contraindication to the use of amyl nitrite, i.e., the presence of glaucoma, for as shown by Wessely,[38] it causes an increase in intra-ocular tension in spite of the lowering of systemic pressure.

CONCLUSIONS

A simple method for quantitating pulmonary cerebral effect time is described, which measures the effective pulmonary ventilation, the circulation time of the left side of the heart, and is affected by variations in the reaction time of the subject.

The normal values in 100 cases are presented.

Its use in determining the degree of failure of circulation of the left side of the heart circuit is thus limited to instances in which no disease of the pulmonary apparatus exists, and in which the reaction time of the subject is not abnormal. Conversely, it may offer an index of the degree of diffuse chronic pulmonary disease in the absence of cardiac disease. When abnormalities are present in both pulmonary and vascular systems, the combined effects may be measured, but without evaluation of the component parts.

I wish to thank Dr. L. N. Katz, under whose supervision this work was done, for suggestions made during the study and preparation of this report.

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REGULATION OF THE RECIPROCAL ACTIVITY OF DESOXYCORTICOSTERONE ACETATE AND SODIUM IN ADDISON'S DISEASE*

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THE early failure to understand the extent to which the physiologic activity of desoxycorticosterone acetate is dependent upon the sodium available in the organism has accounted for most of the complications following its administration to patients with Addison's disease. Six patients with this malady have been treated with the drug† over periods varying from three months to two and one-half years, during which time in each instance there have been wide variations in the amounts of sodium and desoxycorticosterone acetate employed. Patients were judged to be in a state of "adrenal sufficiency," that is, in good clinical condition, when a sense of well-being was subjectively experienced; a gradual gain in weight to normal and its maintenance achieved; a return of blood pressure to satisfactory levels attained; a cardiothoracic ratio between 0.45 and 0.50 observed; and normal values for sodium and potassium noted in both blood serum and urine.

By "trial and error" sodium and desoxycorticosterone acetate have been utilized in 47 different ratios in the above-mentioned six patients for not less than six weeks in any instance, unless severe insufficiency or a complication arose before such a period was completed. The longest time at any single dose level in any one individual has been nine months. When these average daily values for sodium ingested and desoxycorticosterone acetate administered were plotted against each other, it was found that patients in good clinical condition showed a definite reciprocal relationship between sodium intake and desoxycorticosterone acetate requirement which was capable of mathematical expression in terms of hyperbolic curves. Seventy-one per cent of these satisfactory observations lay on or between curves, the constants of which were 37.5 and 45.0, respectively (Fig. 1), while all other observations (29 per cent) in which the clinical condition of the patient was satisfactory were represented by similar parabolic curves, the constants of which ranged from 30 to 37.5, and from 45 to 55, respectively (Fig. 1.)

It is obvious that only portions of the hyperbolas are of practical value. If one uses large quantities of sodium, that is, more than 9 Gm. daily, and correspondingly small doses of desoxycorticosterone acetate, the clinical condition is not as good as though more desoxycorticosterone acetate were employed. If, on

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the other hand, sodium is severely restricted, that is, to 1.5 Gm., and desoxycorticosterone acetate dosage is concomitantly increased, that is, to 30 mg. daily, some "packing" of sodium into the cells probably occurs.[1, 2]

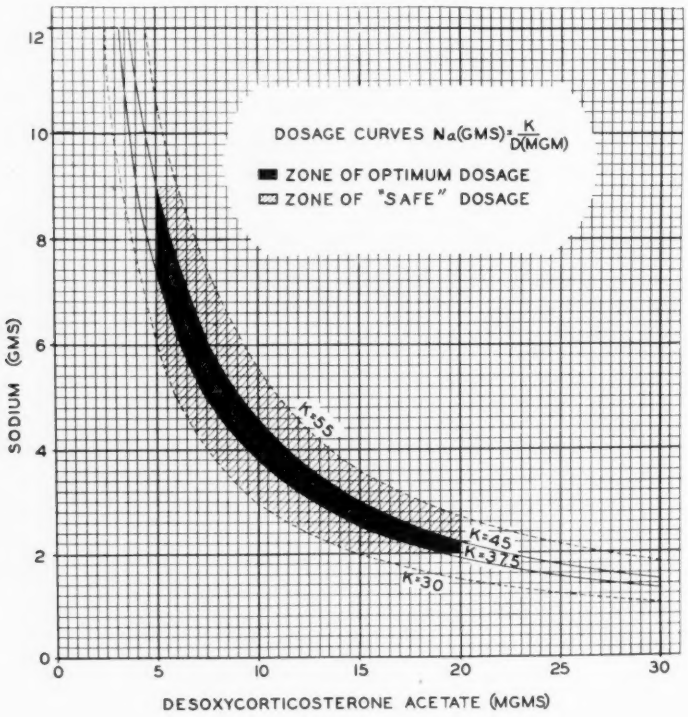

Fig. 1.

The formula $Na\ (Gm.) = \frac{k}{D\ (mg.)}$, where Na equals daily intake of sodium in grams, k is a constant, and D the desoxycorticosterone acetate in milligrams, is applicable to the management of patients with Addison's disease over a definite range of values, suggested by the shaded area in Fig. 1. In our experience, values for the daily intake of sodium should not exceed 9 Gm. or be less than 2 Gm., while less than 5 mg. or more than 20 mg. of desoxycorticosterone acetate daily do not afford as satisfactory relief as those attendant upon amounts between these two figures. Values for k should lie on or between 37.5 and 45. The area delimited by adherence to these figures (black in Fig. 1) may be spoken of as the "area of optimum dosage" in the use of desoxycorticosterone acetate for the management of Addison's disease. Values lying within this space can be apparently applied without danger of disaster from cortical insufficiency or fear of complications directly related to the therapy.

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THE SIGNIFICANCE OF FIBRINOLYSIS IN MECHANISM OF COAGULATION OF BLOOD*

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BY FIBRINOLYSIS is meant an occasionally observed phenomenon which consists in the dissolution of the clot of fibrin formed by the coagulation of whole blood or plasma. This phenomenon has been observed and described by several authors, principally Denis (quoted by Nolf[11]), Dastre,[2] Rulot,[19, 20] Nolf,[11] Hougardy,[4, 5] and Hirose.[18] Since fibrinolysis appears only occasionally in the process of blood coagulation, it is necessary to devise some procedure for obtaining it consistently, in order to be able to investigate it on an experimental basis. The use of chloroform provides such a procedure, as will be shown by the subsequent considerations.

The effect of chloroform on blood coagulation has been investigated principally by Howell,[6] Nolf,[9, 14] Minot,[7] and Bordet.[1] All these authors agree that this substance has a marked effect on the mechanism of blood coagulation; they have observed that chloroform was able to clot oxalated plasma. Nolf emphasized the fibrinolytic power of the serum obtained from oxalated recalcified plasma clotted by chloroform.

As far as fibrinolytic action is concerned, the best procedure to study the effect of chloroform is to observe the serum resulting from oxalated, recalcified plasma clotted by chloroform. Blood is taken by venipuncture from the jugular vein of an unanesthetized, fasting dog. Twenty cubic centimeters of blood are withdrawn in a syringe containing 1 c.c. of 3 per cent sodium oxalate solution. It is then poured into a paraffined centrifuge tube and is centrifuged twice for fifteen minutes at a speed of 3,000 r.p.m. The clear plasma is used to prepare:

1. Normal serum, by simply adding calcium chloride. After coagulation occurs, serum is obtained by centrifugalization.

2. So-called chloroform serum. Recalcified plasma is mixed with $\frac{1}{10}$ its volume of chloroform and is shaken for one minute in a carefully stoppered tube. The tube is maintained at a temperature of 37° C. for one hour. Then chloroform is removed from the mixture, first by centrifugalization and then by evaporation of its supernatant layer in the icebox for sixteen to twenty-four hours. After that time the mixture does not smell of chloroform. Distilled water is added to correct for the loss of water caused by evaporation. The serum is filtered before using.

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In the course of this study, other reagents were used:

Oxalated plasma: as such, or deprived of fibrinogen by heating at 56° C. for two minutes.

Prothrombin: globulin solution prepared from diluted oxalated plasma, precipitated at pH 5.3, following the method of Mellanby.[8] It can be deprived of fibrinogen by heating to 56° C.

Fibrinogen: prepared according to Warner, Brinkhous, and Smith.[23] It was diluted with physiologic saline before being used, until the greatest dilution was obtained which gave, with the addition of thrombin, a coherent clot. This dilution is desirable because it decreases the amount of impurities remaining in the solution.

Thromboplastin: prepared according to Warner, Brinkhous, and Smith.[23]

Oxalated plasma deprived of prothrombin: To remove prothrombin, tricalcium phosphate was used in a thick creamy suspension in saline. Ten parts of oxalated plasma are added with one part of the suspension. After careful stirring, the mixture is warmed to 37° C. for fifteen minutes, and then the phosphate, together with the prothrombin, is removed by centrifugalization. Phosphated plasma does not clot on addition of calcium and thromboplastin. It clots readily on addition of thrombin. It can be deprived of fibrinogen by heating to 56° C.

Thrombin: prepared just before its use by adding calcium and thromboplastin to oxalated plasma or to prothrombin and removing the serum after coagulation.

All the foregoing blood preparations were made from dog blood.

To compare the thrombin content of different sera, successive dilutions of the sera were added to given quantities of fibrinogen. The most dilute amount which produces a clot and thereby contains an appreciable, effective quantity of thrombin is taken as the end point. Sera prepared by various methods—chloroform serum, normal serum—may thus be compared for thrombic activity.

In all the experiments a total volume of 1 c.c. was used. Physiologic saline was used as a diluent. In order to keep the quantity of thrombin tested at a constant known level, an excess of sodium oxalate was added to prevent further increment of thrombin by conversion of any prothrombin which might possibly be present. The experiments were performed at room temperature. Under these conditions the amount of thrombin in chloroform serum and normal serum was compared. Such a comparison is given in Table I.

It appears that chloroform serum contains 300 times as much thrombin as normal serum. Table I shows also that chloroform serum, after having clotted fibrinogen, dissolves it. In the first tube (Table I), the fibrinogen clots in four minutes. The following day the clot had disappeared. The solution was again perfectly clear, and its appearance was absolutely the same as before the occurrence of the clot. An important change, however, had occurred. The solution contained no more fibrinogen. It was impossible to obtain a clot in this solution by adding thrombin. Also, if the content of the first tube is heated to 56° C., fibrinogen precipitate does not appear. The same phenomenon oc-

curred in the two following tubes (Nos. 2 and 3). It occurred in the fourth tube (No. 4), but less intensively. This did not occur with normal serum. The disappearance of the clot is probably due to proteolysis.

TABLE I

COMPARISON OF THE THROMBIN CONTENT OF CHLOROFORM SERUM AND NORMAL SERUM

TUBES	REAGENTS IN CUBIC CENTIMETERS					RESULTS
	SODIUM OXALATE 1%	NaCl 0.90%	FIBRINOGEN	CHLOROFORM SERUM	NORMAL SERUM	
1	0.1	0.7	0.1	0.1		Clot in 4 min. Redissolved after 16 hr.
2	0.1	0.7	0.1	0.01		Clot in 10 min. Redissolved after 8 min.
3	0.1	0.7	0.1	0.003		Clot in 10 min. Redissolved after 20 min.
4	0.1	0.7	0.1	0.001		Clot in 30 min. Half dissolved after 2 hr. 20 min.
5	0.1	0.7	0.1	0.0003		Clot in 1 hr. 10 min. Not redissolved
6	0.1	0.7	0.1	0.0001		Clot in 2 hr. 7 min. Not redissolved
7	0.1	0.7	0.1	0.00003		Clot in 3 hr. 49 min. Not redissolved
8	0.1	0.7	0.1	0.00001		Clot in 10 hr. 57 min. Not redissolved
9	0.1	0.7	0.1		0.1	Clot in 1 hr. 10 min. Not redissolved
10	0.1	0.7	0.1		0.01	Clot in 1 hr. 10 min. Not redissolved
11	0.1	0.7	0.1		0.003	Clot in 2 hr. 20 min. Not redissolved
12	0.1	0.7	0.1		0.001	Clot in 12 hr. Not redissolved
13	0.1	0.7	0.1		0.0003	Clot in 12 hr. Not redissolved
14	0.1	0.7	0.1		0.0001	No clot

If the action of chloroform serum on diluted oxalated plasma is studied, it appears that a solution of the clot does not occur in plasma as markedly as in the fibrinogen solution. Table II shows that dilutions of chloroform serum, which are able to produce fibrinolysis in the fibrinogen solution, do not produce this when acting on diluted plasma. It can be seen in Table III that the quantity of plasma needed to prevent fibrinolysis caused by 0.1 c.c. of chloroform serum is between 0.04 c.c. and 0.01 c.c. (tube 6). The preventive action of plasma on proteolysis is not due to fibrinogen, since this inhibiting effect remains unchanged when plasma is deprived of fibrinogen by heating to 56°. This is apparent in Table IV. It will be shown later that the preventive action is due to the prothrombin of the plasma (Tables IX and X).

In the experiment described above, 0.01 c.c. of chloroform serum causes lysis of the fibrinogen clot after four minutes. Fibrinolysis may occur at an even more rapid rate, and in such cases a well-formed clot would have no time to appear. Table V illustrates such an experiment (tube 2). It can be seen that 0.1 c.c. of chloroform serum causes the production of flakes in the fibrinogen solution. These flakes disappear after two minutes, and thereafter there is no more fibrinogen in the solution. This shows that fibrinolysis may be completed

TABLE II

COMPARATIVE ACTION OF CHLOROFORM SERUM ON FIBRINOGEN AND OXALATED PLASMA

TUBES	REAGENTS IN CUBIC CENTIMETERS					RESULTS
	SODIUM OXALATE 1%	NaCl 0.9%	FIBRINOGEN	OXALATED PLASMA	CHLOROFORM SERUM	
1	0.1	0.7	0.1		0.1	Clot in 2 min. Redissolved after 3 hr.
2	0.1	0.7	0.1		0.01	Clot in 2 min. Redissolved after 3 hr.
3	0.1	0.7	0.1		0.003	Clot in 2 min. Not redissolved
4	0.1	0.7		0.1	0.1	Clot in 2 min. Not redissolved
5	0.1	0.7		0.1	0.01	Clot in 2 min. Not redissolved
6	0.1	0.7		0.1	0.003	Clot in 2 min. Not redissolved

TABLE III

PREVENTIVE ACTION OF PLASMA ON FIBRINOLYSIS CAUSED BY CHLOROFORM SERUM

TUBES	REAGENTS IN CUBIC CENTIMETERS					RESULTS
	SODIUM OXALATE 1%	NaCl 0.9%	FIBRINOGEN	OXALATED PLASMA	CHLOROFORM SERUM	
1	0.1	0.5	0.1	0.2	0.1	Clot in 5 min. Not redissolved
2	0.1	0.6	0.1	0.1	0.1	Clot in 5 min. Not redissolved
3	0.1	0.3	0.1	0.08	0.1	Clot in 5 min. Not redissolved
4	0.1	0.4	0.1	0.06	0.1	Clot in 5 min. Not redissolved
5	0.1	0.5	0.1	0.04	0.1	Clot in 5 min. Not redissolved
6	0.1	0.6	0.1	0.01	0.1	Clot in 5 min. Redissolved after 3 hr.

TABLE IV

ACTION OF PLASMA, DEPRIVED OF FIBRINOGEN, ON FIBRINOLYSIS

TUBES	REAGENTS IN CUBIC CENTIMETERS					RESULTS
	SODIUM OXALATE 1%	NaCl 0.9%	FIBRINOGEN	OXALATED PLASMA DEPRIVED OF FIBRINOGEN	CHLOROFORM SERUM	
1	0.1	0.5	0.1	0.2	0.1	Clot in 5 min. Not redissolved
2	0.1	0.6	0.1	0.1	0.1	Clot in 5 min. Not redissolved
3	0.1	0.3	0.1	0.08	0.1	Clot in 5 min. Not redissolved
4	0.1	0.4	0.1	0.06	0.1	Clot in 5 min. Not redissolved
5	0.1	0.5	0.1	0.04	0.1	Clot in 5 min. Not redissolved
6	0.1	0.6	0.1	0.01	0.1	Clot in 5 min. Redissolved after 3 hr.

TABLE V

FIBRINOLYSIS OCCURRING IMMEDIATELY AFTER COAGULATION

TUBES	REAGENTS IN CUBIC CENTIMETERS				RESULTS
	SODIUM OXALATE 1%	NaCl 0.9%	FIBRINOGEN	CHLOROFORM SERUM	
1	0.1	0.6	0.1	0.2	No change
2	0.1	0.7	0.1	0.1	Flakes after 3 min. Redissolved after 2 min.
3	0.1	0.7	0.1	0.01	Clot in 5 min. Redissolved after 2 hr.

after two minutes. It may even occur so soon that the coagulation could not be observed. The solution of fibrinogen to which chloroform serum is added becomes opalescent and then clear again. No compact clot is formed. The solution, however, contains no more fibrinogen. This phenomenon is illustrated in Table V (tube 1). Nothing happens in the tube to which 0.2 c.c. of chloroform serum is added, but in the tubes to which is added 0.01 c.c. of the serum, coagulation occurs, soon followed by proteolysis. In the first tube, fibrinogen disappears, fibrinolysis having set in so fast that the fibrin flakes, if formed at all, are dissolved as soon as they are formed. At this dilution chloroform serum seems to have no clotting power. Moreover, a superficial observation would lead one to believe that it has an anticlotting power. However, this is not so, since fibrinogen originally present has disappeared. It is, therefore, impossible to obtain coagulation in the first tube. The disappearance of fibrinogen is the real cause for the incoagulability. Moreover, this interpretation is confirmed by the fact that the same quantity of serum, which produces no apparent coagulation in the fibrinogen, does clot oxalated diluted plasma. Fibrinolysis does not occur in plasma as it does in the fibrinogen mixture, probably because of some antilytic agents in the former.

Fibrinolysis usually occurs without apparent coagulation if serum kept in contact with chloroform for twenty-four hours is used. This is not usually the case with serum kept in contact with chloroform for only one hour. The fibrinolytic property is increased when the contact time with chloroform is prolonged, provided that this time does not exceed forty-eight hours (Table VI).

TABLE VI

COMPARISON OF THE FIBRINOLYTIC POWER OF CHLOROFORM SERUM AFTER ONE HOUR OF CONTACT WITH CHLOROFORM AND AFTER TWENTY-FOUR HOURS

TUBES	SODIUM OXALATE 1%	NaCl 0.9%	FIBRINOGEN	CHLOROFORM SERUM AFTER ONE HOUR OF CONTACT	CHLOROFORM SERUM AFTER 24 HOURS OF CONTACT	RESULTS
1	0.1	0.7	0.1	0.1		Clot in 5 min. Redissolved after 10 min.
2	0.1	0.7	0.1	0.01		Clot in 10 min. Redissolved after 30 min.
3	0.1	0.7	0.1	0.001		Clot in 30 min. Not redissolved
4	0.1	0.7	0.1		0.1	No change (proteolysis without coagulation)
5	0.1	0.7	0.1		0.01	No change (proteolysis without coagulation)
6	0.1	0.7	0.1		0.001	Clot in 45 min. Not redissolved

In order to investigate further the relationship of fibrinolysis to the mechanism of blood coagulation, chloroform serum produced by allowing serum to remain in contact with chloroform for one hour at 37° C. and for twenty-four hours at room temperature, was used in the following experiments. Hereafter the expression "chloroform serum" will always refer to this particular twenty-four-hour-old serum.

It has been shown (Table V) that oxalated plasma contains antilytic substances. The substances contained in plasma, however, are not the only ones showing an antilytic effect. Thromboplastin exhibits it also. It can be seen in Table VII (tube 3) that 0.1 c.c. of thromboplastin, diluted ten times, counteracts the fibrinolytic effect of a quantity of chloroform serum able to fibrinolyze the fibrinogen without producing clot formation (tube 5). Consequently, if two identical mixtures of oxalated fibrinogen and oxalated chloroform serum are used, thromboplastin added to one of them will cause a clot to appear while the other one remains fluid and undergoes fibrinolysis without coagulation. This antilytic effect of thromboplastin is very powerful.

TABLE VII

ANTILYTIC EFFECT OF THROMBOPLASTIN

TUBES	OXALATED CHLOROFORM SERUM	NaCl 0.9%	OXALATED FIBRINOGEN	THROMBOPLASTIN (DILUTED 10 TIMES)	RESULTS
1	0.1	0.5	0.1	0.3	Clot in 5 min. Not redissolved.
2	0.1	0.6	0.1	0.2	Clot in 5 min. Not redissolved.
3	0.1	0.7	0.1	0.1	Clot in 5 min. Redissolved after 10 hr.
4	0.1	0.7	0.1	0.03	Proteolysis without coagulation
5	0.1	0.8	0.1	0	Proteolysis without coagulation

In order to ascertain more accurately the fibrinolytic action of chloroform serum, the following procedure was undertaken: Chloroform serum was diluted with 15 volumes of distilled water and, with the use of N/10 acetic acid, mixtures of varying pH were obtained. The globulin precipitate obtained in every case was dissolved in saline, and its action was tested on fibrinogen and oxalated plasma. Only the product obtained at pH 6 showed any activity comparable with that of the chloroform serum which produced it. In addition, it revealed some interesting properties which explain the action of chloroform serum.

The detailed method of preparation was the following: Chloroform serum is diluted with 15 volumes of cold, distilled water in order to lower the salt concentration below 1 per thousand. Decinormal acetic acid is added until pH 6 is reached. The slightly turbid solution is centrifuged, and the precipitate is dissolved in a volume of saline equal to one-third that of the chloroform serum from which it was obtained. Sodium oxalate is added in order to obtain a concentration of soluble oxalate of 0.15 per cent. The solution is centrifuged, and its pH is adjusted to neutrality. This solution is called *solution A* in the experiments to be detailed.

The properties of solution A are:

1. It is not a solution of thrombin, since at no concentration does it clot fibrinogen. Unlike chloroform serum, of which a concentration can always be found which clots fibrinogen and dissolves it afterwards, every active concentration of solution A fibrinolyzes fibrinogen without clotting it. Dilute quantities of solution A leave it unchanged. Table VIII shows the difference in the effects of chloroform serum and of solution A (prepared from the chloroform serum) on fibrinogen.

TABLE VIII

COMPARISON OF THE EFFECT OF CHLOROFORM SERUM AND OF SOLUTION A ON FIBRINOGEN

TUBES	OXALATED CHLOROFORM SERUM	OXALATED SOLUTION A	OXALATED FIBRINOGEN	Nacl 0.9%	RESULTS
1	0.1		0.1	0.7	Proteolysis without coagulation
2	0.01		0.1	0.7	Proteolysis without coagulation
3	0.001		0.1	0.7	Clot in 20 min. Redissolved after 10 min.
4	0.0001		0.1	0.7	No clot. No proteolysis
5	0.00001		0.1	0.7	No clot. No proteolysis
6		0.4	0.1	0.5	Proteolysis without coagulation
7		0.2	0.1	0.7	Proteolysis without coagulation
8		0.1	0.1	0.8	Proteolysis without coagulation
9		0.06	0.1	0.7	No clot. No proteolysis
10		0.03	0.1	0.7	No clot. No proteolysis

TABLE IX

ACTION OF SOLUTION A ON OXALATED PLASMA

TUBES	OXALATED PLASMA DEPRIVED OF FIBRINOGEN	OXALATED FIBRINOGEN	OXALATED SOLUTION A	Nacl 0.9%	RESULTS
1	0.1	0.1	0.5	0.3	Clot in 15 min. Redissolved after 2 hr.
2	0.08	0.1	0.5	0.1	Clot in 15 min. Redissolved after 1 hr.
3	0.06	0.1	0.5	0.2	Proteolysis without coagulation
4	0.04	0.1	0.5	0.3	Proteolysis without coagulation
5	0.02	0.1	0.5	0.3	Proteolysis without coagulation

TABLE X

ACTION OF SOLUTION A ON PHOSPHATED PLASMA

TUBES	REAGENTS IN CUBIC CENTIMETERS			RESULTS
	PHOSPHATED PLASMA	Nacl 0.9%	OXALATED SOLUTION A	
1	0.1	0.1	0.4	Proteolysis without coagulation
2	0.1	0.3	0.2	Proteolysis without coagulation
3	0.1	0.5	0.05	No proteolysis. No coagulation
4	0.1	0.7	0.01	No proteolysis. No coagulation

TABLE XI

ACTION OF SOLUTION A ON FIBRINOGEN TO WHICH THROMBOPLASTIN IS ADDED

TUBES	OXALATED FIBRINOGEN	THROMBOPLASTIN	OXALATED SOLUTION A	Nacl 0.9%	RESULTS
1	0.1	0.02	0.4	0.3	Proteolysis without coagulation
2	0.1	0.01	0.4	0.4	Proteolysis without coagulation
3	0.1	0.003	0.4	0.4	Proteolysis without coagulation
4	0.1	0.001	0.4	0.4	Proteolysis without coagulation

2. Added to oxalated plasma, solution A has the same action as chloroform serum. It clots oxalated plasma, provided there is a sufficient amount of the latter (Table IX, tubes 1 and 2). If the quantity of plasma is too small, solution A fibrinolyzes fibrinogen without clotting it, as shown in Table IX, tube 3.

3. Added to oxalated plasma deprived of prothrombin (phosphated plasma), solution A never clots it, but added in sufficient quantity solution A fibrinolyzes the fibrinogen of phosphated plasma (Table X, tubes 1 and 2). Added in smaller quantities, it leaves it intact, as shown in Table X, tubes 3 and 4.

4. If thromboplastin is added to fibrinogen, solution A produces no clot, in contrast to chloroform serum the fibrinolytic power of which, as stated previously, can be transformed into clotting power by means of thromboplastin (Table XI).

5. From the foregoing experiments it may be concluded that solution A is not prothrombin. Prothrombin has no clotting effect in the absence of calcium.

6. Solution A is not thromboplastin because thromboplastin never clots oxalated plasma.

In summary, it may be said that solution A has an enzymelike, and probably a proteolytic effect on fibrinogen. Its activity, with respect to the phenomenon of blood coagulation, presents the following features: it clots oxalated plasma. Unlike chloroform serum, its mother substance, solution A never clots plasma deprived of prothrombin, nor does it coagulate fibrinogen to which thromboplastin is added. The clotting activity of solution A is probably due to its action on prothrombin, which is converted into thrombin even in the presence of an excess of sodium oxalate without participation of calcium platelets or thromboplastin.

There is a striking similarity of action between the enzymelike substance contained in solution A and trypsin. The effect of trypsin on blood coagulation was studied principally by Eagle and Harris.[3] According to these authors, trypsin, like solution A, lyzes fibrinogen without clotting it, but it has a strong clotting action on oxalated plasma without the participation of calcium, platelets, or thromboplastin. Added to prothrombin, optimum quantities of trypsin change it into thrombin. Larger quantities fail to do so since they bring about the destruction of prothrombin. The same thing happens when trypsin is allowed to remain in contact with prothrombin for a long time. Both prothrombin and the thrombin which is formed are destroyed. Eagle and Harris conclude that trypsin acts in the mechanism of blood coagulation like the system calcium platelets or calcium thromboplastin does. The theory of blood coagulation, adopted by Eagle and Harris, according to which thrombin results from the interaction between prothrombin and thromboplastin plus calcium, permits no other conclusion.

In order to investigate further the identity of the action of trypsin and the enzyme contained in solution A, the effect of solution A on the prothrombin solution, prepared as described above, was studied. Varying quantities of solution A were added to 0.5 c.c. of oxalated prothrombin. They were allowed to

interact at 37° C. during different periods of time. The thrombin produced in every case was tested on fibrinogen, and the clotting time was recorded. To rule out an eventual spontaneous formation of thrombin from prothrombin, a control was made by allowing pure prothrombin to remain at 37° C. for the time of the experiment, and testing it on fibrinogen. The experiment was carried out at pH 6.7, 0.5 c.c. of phosphate buffer being added to each sample. The results (Chart 1) show that the activation of prothrombin into thrombin by solution A is soon followed by inactivation of both the prothrombin and the thrombin formed. This is consistent with the results of Eagle and Harris with trypsin.

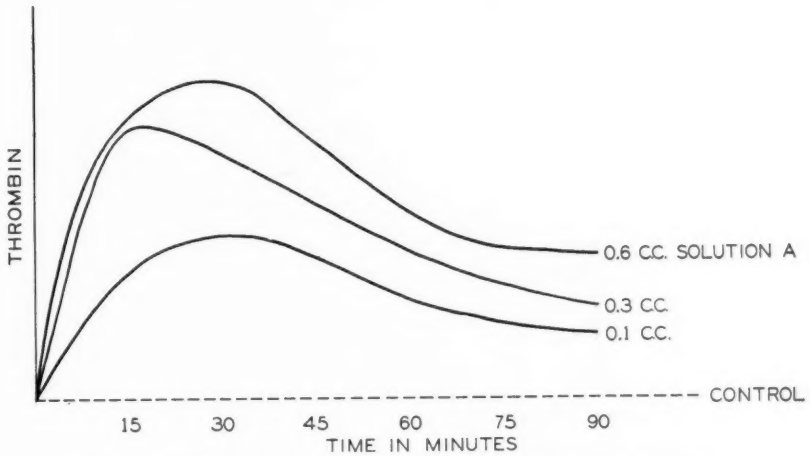

Chart 1.—Production of thrombin from prothrombin and solution A.

The same procedure which furnished solution A can be applied to a chloroform serum, which is characterized by contact of the serum and chloroform for less than twenty-four hours. In this case the solution obtained has the properties of thrombin which are more marked the shorter the time of contact with chloroform.

The isoelectric precipitation at pH 6 of diluted chloroform serum is not the only means to isolate the active fibrinolytic principle from this serum. A highly satisfactory process consists in dialyzing chloroform serum in cellophane against running tap water below 10° for three days; the precipitate of euglobulin formed is treated as the precipitate obtained by isoelectric precipitation. Both solutions, obtained either by precipitation at pH 6 or by dialysis, exhibit exactly the same properties and may, therefore, be assumed to contain the same principle.

DISCUSSION

The experimental data indicate that chloroform serum prepared from the blood of dogs after twenty-four hours of contact with chloroform exhibits properties different from that obtained after only one hour of contact. While chloroform serum obtained after only one hour of contact with chloroform coagulates fibrinogen with subsequent fibrinolysis, that obtained from twenty-four hours contact in the same concentration produces complete fibrinolysis

without preliminary coagulation. Very low concentration of chloroform serum obtained after twenty-four hours contact will clot fibrinogen. The clot, however, disappears very rapidly.

The saline solution of the globulin fraction derived from chloroform serum after twenty-four hours (solution A), prepared by isoelectric precipitation at pH 6, or by dialysis, differs from chloroform serum itself in several particulars. While solution A can coagulate oxalated plasma, it cannot, unlike chloroform serum, in any concentration coagulate phosphated plasma or fibrinogen to which thromboplastin has been added or pure fibrinogen itself. Prothrombin must be added to fibrinogen solutions before coagulation takes place.

Solution A, however, does produce fibrinolysis but does so without any preliminary clotting activity. Chloroform serum does not require the presence of added prothrombin because it presumably already contains some of this substance, in either free or combined form. In certain concentrations, therefore, chloroform serum acts like thrombin, since it can produce coagulation of pure fibrinogen.

With respect to blood coagulation, solution A has enzymelike characteristics and resembles much in detail the action of trypsin reported by Eagle and Harris.[3] So marked was this resemblance that one wonders whether this enzymelike material could be of pancreatic origin. Pancreatectomized dogs, the pancreatectomy being confirmed by post mortem and microscopic verification,[22] from whose blood chloroform serum was made at intervals over several months, disproved this hypothesis. The characteristics of the chloroform serum remained unchanged.

Eagle and Harris accept the two-stage theory of blood coagulation whereby:

$$1.\ \text{Prothrombin} + \text{Ca}^{++} + \left\{ \begin{array}{c} \text{Platelets} \\ \text{or} \\ \text{Thromboplastin} \end{array} \right\} \rightarrow \text{Thrombin.}$$

$$2.\ \text{Thrombin} + \text{Fibrinogen} \rightarrow \text{Fibrin (clot).}$$

From their studies on trypsin they propose an enzymatic modification of this reaction as follows:

1. Prothrombin + Trypsin → Thrombin.
2. Thrombin + Fibrinogen → Fibrin (clot).

From this substitution the conclusion must be drawn that trypsin replaces calcium, platelets, and thromboplastin in the blood coagulation reaction.

The possibility that trypsin, a substance not yet identified with any blood constituent, having any connection with blood coagulation, seemed remote. The evidence that solution A resembles trypsin in many of its reactions is important, since solution A is obviously of plasma origin, requiring only the addition of chloroform to oxalated recalcified plasma to activate it.

However, the experimental evidence presented in this paper does not permit the identification of solution A with any of the substances in the coagulation reaction presented by Eagle and Harris. It contains neither thromboplastin nor fibrinogen, and its calcium ion content, if any, must be infinitesimal. Certainly

no preformed thromboplastin was present since the parent plasmas showed no production of fibrin even after standing for eight days. Neither can solution A be said to be thrombin since it does not in pure form clot fibrinogen.

If it can be said that trypsin, a substance foreign to the blood, can replace calcium plus platelets or thromboplastin in the blood coagulation reaction, it would logically follow from the experimental data, and from the fact that solution A is a true blood derivative that it too can replace the calcium thromboplastin platelet system.

Solution A is of plasma origin and can produce thrombin from prothrombin. It is highly probable, therefore, that it plays an essential role in the blood coagulation reaction. Its role is of such a nature that it would appear that calcium thromboplastin or platelets are no longer necessary in the coagulation reaction or play only a minor role.

One may go even farther than this and speculate whether the theoretical outline of blood coagulation outlined above can any longer be true, since it obviously does not lend itself to the inclusion of the existence of an enzyme theory which the evidence of this communication would appear to indicate.

The evidence for the enzymelike character of this plasma derivative may be briefly summarized. It lyzes fibrinogen, except in the presence of prothrombin, when coagulation intervenes followed by fibrinolysis. Neither calcium nor thromboplastin plays any direct role in this reaction. Thromboplastin can play a secondary role, as shown by the fact that the addition of *chloroform serum* in an amount that would normally lyze fibrinogen without coagulation, may be made to coagulate the fibrinogen if thromboplastin is added. However, thromboplastin does not have even this effect on *solution A*. It can augment the effect of prothrombin in this regard but alone has no effect on solution A.

As remarked earlier in the discussion, chloroform serum prepared after one hour's contact with chloroform differs in essential characteristics from that prepared after contact for twenty-four hours. A discussion of the reasons for the difference seems appropriate.

During the initial shaking with chloroform, thrombin is immediately formed and in large concentration, as shown in Table I. Fibrin is also precipitated. As time goes on the fibrin undergoes fibrinolysis due to the apparent proteolytic properties now present in chloroform serum. At the end of twenty-four hours, at which time the chloroform is removed by centrifuging and aeration, there is no longer any fibrin present.

These facts are interpreted by reasoning that under the influence of chloroform an enzymelike substance is produced which forms thrombin from prothrombin in the presence of calcium ion. The result of this reaction is fibrin formation. During the subsequent twenty-four hours the enzymelike substance proteolyzes both prothrombin, fibrinogen, if any is still present, and fibrin. Table VIII and Chart 1 clearly indicate that the enzymelike material is able to produce both of these effects. With the disappearance of prothrombin the enzyme has no longer any ability to coagulate fibrinogen, but becomes purely fibrinolytic unless prothrombin is subsequently restored. The last traces of prothrombin are removed when solution A is formed by isoelectric precipitation or dialysis of the chloroform serum.

The possibility that thrombin itself may contain as part of that complex the enzymelike material described in this paper cannot be excluded by the experimental data here presented. The failure of certain thrombin preparations[21] to display any such fibrinolytic activity would then be explained by the presence of thromboplastin as an impurity in such preparations. The data show that thromboplastin when present can inhibit fibrinolysis. The fibrinolytic properties of thrombin previously described by Nolf[13] and ascribed to the presence of one of its constituent substances thrombozyme may indicate a possible identity between thrombozyme and solution A.

The fact that the enzymelike substance contained in chloroform serum is precipitated at pH 6 and by dialysis suggests the possibility of a similarity between the active principle of solution A and the plasma substance acting on hemophilic blood, investigated by Patek, Stetson, and Taylor.[15-17] Should this be the case, hemophilia would be due to a lack of proteolytic fibrinolytic-clotting enzyme in the plasma.

SUMMARY

1. A method is devised for obtaining from oxalated plasma a proteolytic enzyme active in blood coagulation.

2. Similar to trypsin, this enzyme participates in the mechanism of blood coagulation by changing prothrombin into thrombin without calcium, thromboplastin, or platelets.

3. The proteolytic action of the enzyme on fibrinogen is counteracted by prothrombin. The action of prothrombin is reinforced in this respect by thromboplastin. Phosphated plasma and thromboplastin, without prothrombin, have no antilytic power. Prothrombin permits the enzyme to manifest its action by a clotting effect.

4. The antilytic action of thromboplastin explains why the proteolytic properties of thrombin were not found by many workers since their thrombin was prepared by means of thromboplastin.

5. The fact that the enzyme is extracted from plasma and that it reacts with prothrombin to form thrombin without intervention of calcium or thromboplastin is good evidence that the enzyme plays a primary role in the mechanism of normal blood coagulation. This role is discussed.

6. It is suggested that this enzyme is the substance, the absence of which causes hemophilia.

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THE EFFECTS OF POLYVINYL ALCOHOL INJECTIONS ON BLOOD AND TISSUES*

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POLYVINYL alcohol is a synthetic chemical.† Its colloidal nature, water solubility, and inertness suggested that it may prove valuable as a drug for increasing blood colloids and thus osmotic pressure and blood volume. Lowered oncotic pressure due to loss of plasma protein plays a large part in the production of nephrotic edema. Such substances as serum transfusion and intravenous gum acacia solution have been used successfully to counteract this lowered oncotic pressure. Effects of intravenous acacia were studied in this laboratory.[1]

Hueper,[2] working with rats and rabbits, reports the pathology produced by oral and intravenous administration of repeated small doses of polyvinyl alcohol. This paper reports the results of a single massive dose of polyvinyl alcohol on kidney tissue and the blood of normal dogs.

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†Developed by DuPont Company.

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Polyvinyl alcohol used in this work technically referred to as "RH-391 type B." A 5 per cent solution of this chemical in physiologic saline, warmed to body temperature, was administered to normal dogs intravenously, in one massive dose of 1 Gm. of the chemical per kilogram of body weight. Blood samples were taken before and at definite time intervals after the injection. Sedimentation rate (Winthrop method), cell volume, red and white blood cell counts, oxygen and carbon dioxide content, and oxygen capacity were determined on these blood samples. Blood and plasma smears were tested for the presence of polyvinyl alcohol by Lugol's solution as described by Hueper. Washed cells were also tested for the chemical before and after hemolysis and urine was also tested for the presence of the chemical. Polyvinyl alcohol particles stain blue with Lugol's solution. Animals were observed for clinical symptoms of any ill effects, and the tissues of sacrificed animals were grossly and microscopically examined for damage caused by this chemical.

TABLE I

BLOOD DETERMINATIONS ON A HEALTHY DOG BEFORE AND AFTER INTRAVENOUS INJECTIONS OF POLYVINYL ALCOHOL*

	BEFORE INJECTION	AFTER INJECTION				
		½ HR.	1 HR.	4 HR.	24 HR.	7 DAYS
Hemoglobin (per cent)	127	82	87	110	119	112
White blood cells (thousands)	14.6	5.6	5.8	12.9	19.4	9.4
Red blood cells (millions)	6.8	5.2	4.9	5.6	6.3	6.0
Cell volume (per cent)	71.9	41.4	42.5	45.8	44.7	59.9
Sedimentation rate, millimeters in						
15 minutes	0	53	40	43	5	3
30 minutes	0	57	53	50	14	3
45 minutes	1	58	55	53	40	31
60 minutes	1	60	56	53	43	37
CO_2 content (volume per cent)	53.2	54.1	52.2	55.1	40.9	46.6
O_2 content (volume per cent)	20.2	12.2	13.5	13.5	18.5	18.5

*A single dose of 1 Gm. per kilogram of body weight as 5 per cent solution in physiologic saline.

TABLE II

BLOOD DETERMINATION ON HEALTHY DOGS AFTER INJECTION OF POLYVINYL ALCOHOL

(Averages of all experiments)

	DAYS AFTER INJECTION				
	7	14	20	30	45
Hemoglobin (per cent)	100	115	110	100	120
White blood cells (thousands)	15.0	10.5	11.5	12.9	12.0
Red blood cells (millions)	6.2	5.9	7.3	6.6	6.6
Cell volume (per cent)	43.6	46.3	46.3	45.3	47.9
CO_2 content (volume per cent)	52.5	51.5	52.3	46.6	50.4
O_2 content	14.3	15.5	14.9	14.6	16.3
Sedimentation rate	Stays increased even after forty-five days				

Eight dogs were used in these experiments. Injections were repeated on two dogs a second time. One was given a second dose of 0.5 Gm. of the chemical per kilogram of body weight twenty-one days after the first injection, and the other was given 1.0 Gm. per kilogram sixty days after the first injection. As the results of all the experiments show a similar trend, observations on only one

dog are recorded in the first table, and averages of all observations are given in the second.

Blood samples taken half an hour after the injection of polyvinyl alcohol show a marked decrease in all blood values, a dilution effect. The drop in the white blood cell count is relatively greater than the drop in other blood constituents. Unlike other blood values, the white blood cell count rises above normal after the initial drop and then comes back to normal gradually. Rise and

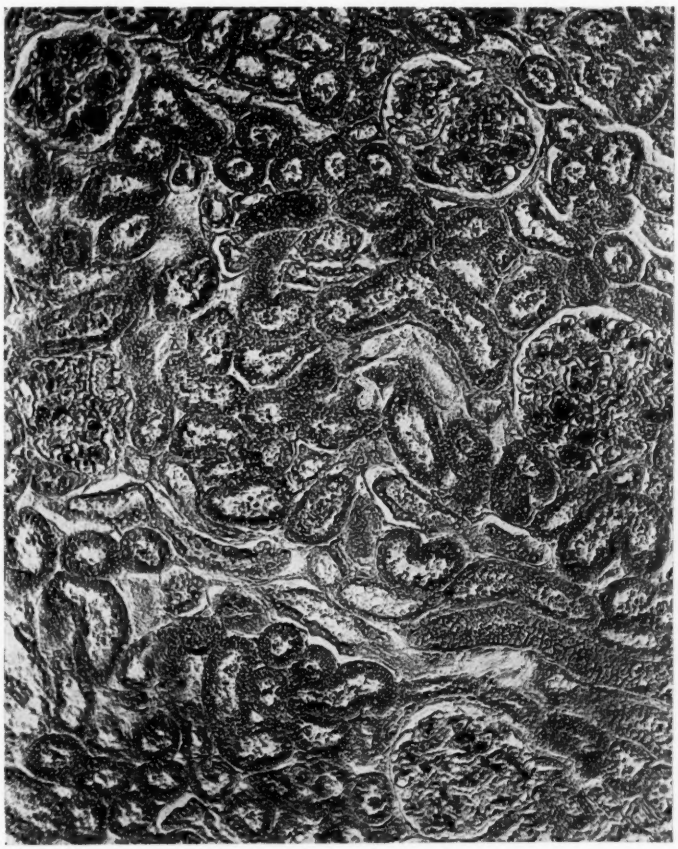

Fig. 1.—Early sections.

fall in hemoglobin, red blood cells, cell volume, and oxygen content values follow each other very closely. Carbon dioxide content of the blood falls only after twenty-four hours after the injection. Sedimentation rate is very rapid half an hour after the injection and does not even approach normal at the end of forty-five days after the injection. All other blood values return to, or approach, normal within one to three weeks after the injection. Oxygen-combining power of the hemoglobin as determined by oxygen capacity per unit of hemoglobin is undiminished.

Staining tests show that polyvinyl alcohol can be traced in the blood for forty to fifty days, and gives very strong and unmistakable reaction up to thirty

days after its injection. This chemical circulates in the plasma and does not enter the blood cells. It is excreted in urine, but to a very small degree. It accumulates in the liver, spleen, and kidney.

One dog became ill, refused to eat, vomited, developed diarrhea and a cold, lost weight, and died two weeks after the injection. The second dog lost his appetite and began to lose weight shortly after the injection, and was sacrificed. The remainder of the dogs showed an initial loss of appetite and weight, but returned to normal in a few days and remained so for the period of observation. The second injections were tolerated as well as the first.

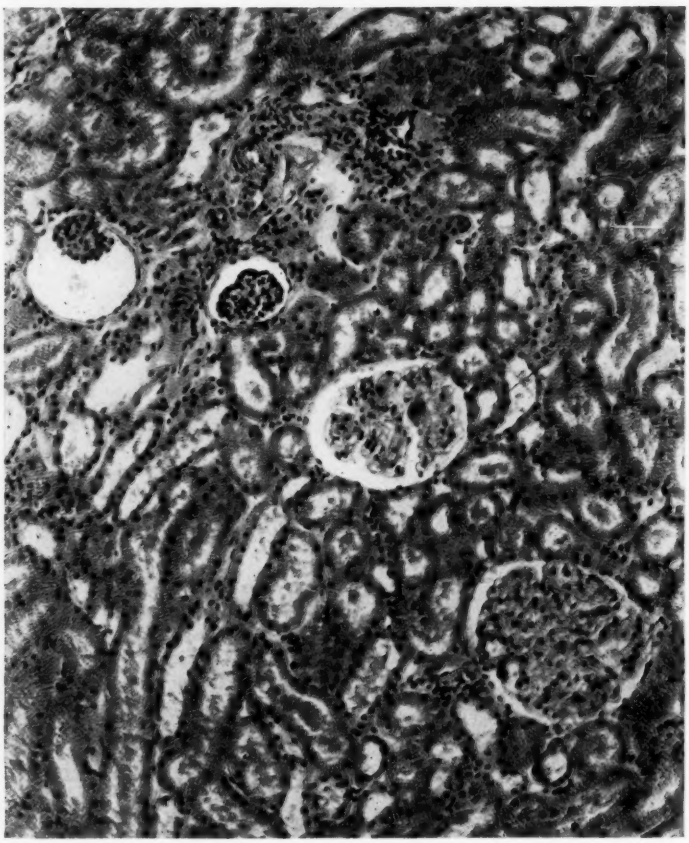

Fig. 2.—After second dose.

Tissue examination showed rather marked tubular degeneration, damage to glomerular tufts, and endothelial swelling filled with polyvinyl alcohol (Fig. 1). The tubular lumina and epithelial lining also contained the chemical. Liver and spleen tissue also showed accumulation of polyvinyl alcohol. In the tissues of the dogs given second injections, many glomerular tufts were only one-quarter normal size due to fibrosis and round-cell infiltration (Fig. 2). Hueper, working with rats and rabbits, found similar damage to tissues after several subcutaneous or intravenous injections of polyvinyl alcohol in small doses.

Although polyvinyl alcohol is an inert chemical, and animals receiving it even in massive doses seem to be normal after initial loss of weight and appetite, blood and tissue examinations show that the animal body is unable to break up and eliminate this compound, thus causing its accumulation in tissues and tissue damage, the kidney in particular. This makes it unsuitable and probably dangerous as a therapeutic agent.

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THE TREATMENT OF ARTHRITIS BY ELECTROPYREXIA INCLUDING SOME PHYSIOLOGIC STUDIES DURING FEVER THERAPY*

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NUMEROUS reports have appeared since our original communication[1, 2] evaluating artificial fever in the treatment of chronic arthritis. Marked differences of opinion exist as to the merits of this therapy.[3-11] Our study gives the results obtained with electropyrexia in 27 patients suffering from chronic infectious arthritis.

METHODS

The patients ranged in age from 17 to 68 years and were selected from the arthritis clinic of Northwestern University Medical School. Each patient was examined in the general medical clinic, and was followed by a re-examination in the arthritis clinic according to a definite routine. This ruled out many vague syndromes simulating arthritis, and at the same time gave us an opportunity to determine the patients' probable ability to withstand the treatment. Electropyrexia was advised after the routine measures of management had been tried without any improvement.

The treatment, given once a week, consisted of four hours of fever between 104° F. and 104.6° F. An effort was made to keep the temperature as close to 104° F. as possible. Each patient received from six to eight treatments.

At the time of the physical examination of the patients, a quantitative chemical analysis for total and fractional proteins was made, in addition to the usual laboratory test.

The patient's temperature was elevated by using a fever cabinet in combination with an inductotherm. Rectal temperature was read with an indicating

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pyrometer and recorded every fifteen minutes, together with the pulse rate and respiration. Fluids were administered to the patients in the form of 0.4 per cent sodium chloride solution. The fluid intake was limited to 250 c.c. for each fifteen-minute period unless more could be given without causing distress. In this way nausea or vomiting was seldom encountered.

None of our patients was given sedatives.

At the beginning of each treatment when the patient's rectal temperature reached 104° F., when this temperature had been maintained for four hours, and, finally, when the patient's rectal temperature had returned to normal the following physiologic tests were undertaken:

1. Blood pH determinations by means of the Dole glass electrode were made on 10 patients.
2. Blood density determinations, using the Barbour and Hamilton[12] falling-drop method, were made on 11 patients.
3. Ascorbic acid concentration of the blood was determined in 18 patients by the method of Farmer and Abt.[13]
4. Circulatory changes were measured in the ring finger of the left hand by means of the Johnson air conduction plethysmograph on 12 patients.
5. Blood pressure was recorded before and after each treatment for 10 patients.

TABLE I

Markedly improved	6 patients (22%)
Moderately improved	6 patients (22%)
Slightly improved	9 patients (33%)
Unimproved	6 patients (22%)
	27

RESULTS

Following fever therapy, the patients returned to the arthritis clinic where the clinicians of that department evaluated the improvement. The results are tabulated in Table I. The criteria used in evaluating improvement in this series included the patient's statement of relief of pain and stiffness. The degree of fatigue was less, and its occurrence was not so frequent. The objective evidence consisted of decreased swelling, increased motion of joints without pain, and gain in weight. Improvement was graded according to the period of time relief persisted.

Slight improvement consisted of temporary improvement, with decreased pain and stiffness in joints for a variable period of time. Moderate improvement consisted of decreased pain and stiffness, decreased swelling, and increased motion in the joints for three months or longer. Marked improvement consisted of decreased pain, stiffness and swelling, with marked increase in joint motion; patient able to return to work. The period of relief lasted for one year or longer.

It will be noted (Table I) that in only six (22 per cent) of the group were the results sufficiently striking over a period of one year or longer to be classified as markedly improved. In no case in the group was a complete permanent result obtained so that the designation "cured" could be used.

TABLE II

LABORATORY DATA

A. Specific Gravity of Blood

	ELAPSED TIME HOURS	AVERAGE VALUE	2 σ DIFFERENCE $\pm$	CHANGE $\pm$ FROM CONTROL	NO. OF OBSERVATIONS
Control	0	1.0536	-		30
Temperature reached 104° F.	2	1.0555	0.0018	0.0019	
Temperature four hours at 104°-104.6° F.	6	1.0552	0.0018	0.0016	
Temperature normal	8	1.0544	0.002	0.0008	

B. Ascorbic Acid Blood Concentration mg./100 c.c.

Control	0	0.62	-	-	77
Temperature reached 104° F.	2	0.68	0.104	0.06	
Temperature four hours at 104°-104.6° F.	6	0.66	0.106	0.04	
Temperature normal	8	0.64	0.088	0.02	

C. Blood pH

Control	0	7.41	-	-	33
Temperature reached 104° F.	2	7.55	0.038	0.14	
Temperature four hours at 104°-104.6° F.	6	7.52	0.0396	0.11	
Temperature normal	8	7.418	0.028	0.003	

D. Blood Pressure Changes

	AVERAGES		DIFFERENCE	2 σ DIFFERENCE $\pm$		OBSERVATIONS
	BEFORE	AFTER				
A. Systolic pressure	129	108	-21	7		24
B. Diastolic pressure	83	69	-14	6.4		
C. Pulse pressure	46	39	- 7	-		

In Table II data of the laboratory studies are given. The average blood pH for the group was 7.415 for the control period, 7.55 at a temperature of 104° F., 7.52 four hours later, and 7.418 when the temperature dropped to normal. This shows an uncompensated alkalosis during the febrile period.

The greatest change in the blood density occurred during the induction of the fever, but inasmuch as the only significant figure was in the fourth decimal place, we have considered the change as insignificant.

Fig. 1 shows the typical finger volume changes of the average patient secured by means of the plethysmograph. The photographic record, Fig. 1, clearly shows the magnitude of the changes found. The maximum response occurred between a temperature of 103° F. and 104° F. Occasionally the maximum response was recorded at 102° F.

No significant changes in the ascorbic acid concentration of the blood plasma were found which could be attributed to the influence of electropyrexia.

The average of 26 determinations of total serum protein, albumin, and serum globulin of the blood prior to treatment are shown in Table III. The total serum protein of the blood ranged from 6.02 per cent to 9.2 per cent. The great majority (18) were within the accepted standard range of 6.0 to 8.0 Gm. per 100 c.c. Eight patients ranged from 8.1 to 9.2 per cent. Three patients showed a higher percentage of serum albumin than the normal range of 3.6 to 5.4 per cent. The serum globulin was lower than the normal range of 1.5 to 3.4 in four patients, and was higher in four. The albumin-globulin ratio was reversed in two patients. The average ratio for the 26 patients was 1.9. One patient was below the accepted normal range of 1.2 to 2.6, and six were above. The average value for fibrinogen was 0.42.

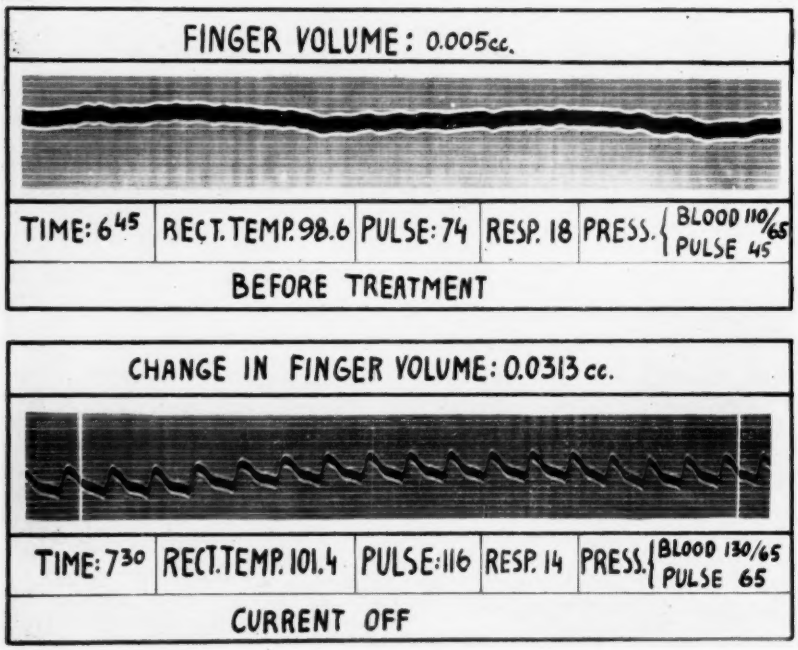

Fig. 1A.

Fig. 1*A*, *B*.—The effect of artificial fever on the blood volume of the finger as measured by the air conduction plethysmograph.

The systolic blood pressure averaged 129 mm. Hg before treatment, and the diastolic pressure averaged 83 mm. Hg. After treatment both systolic and diastolic pressures had fallen to 108 and 69 mm. Hg, respectively (Table II *D*). These were significant changes as can be seen by referring to Table II.

PATIENT REACTIONS

The majority of the patients tolerated the treatment quite well. The complaint of headache was not infrequent. Occasionally a patient had paraesthesia of both hands, and dizziness. One patient of this series vomited. An unlisted patient at the completion of his first treatment suddenly failed to respond to

questions, and then became irrational. There was a brief period of paralysis of the right arm and right leg. The left nasolabial fold was erradicated, and the mouth was drawn down on the left side. After a period of thirty minutes the patient was able to respond to questions but was still confused. Still another patient, L. B., developed an auricular fibrillation, was nauseated, had marked circumoral pallor, cold skin, and complained of faintness and abdominal pain. This instance of shock developed during the sixth and last treatment.

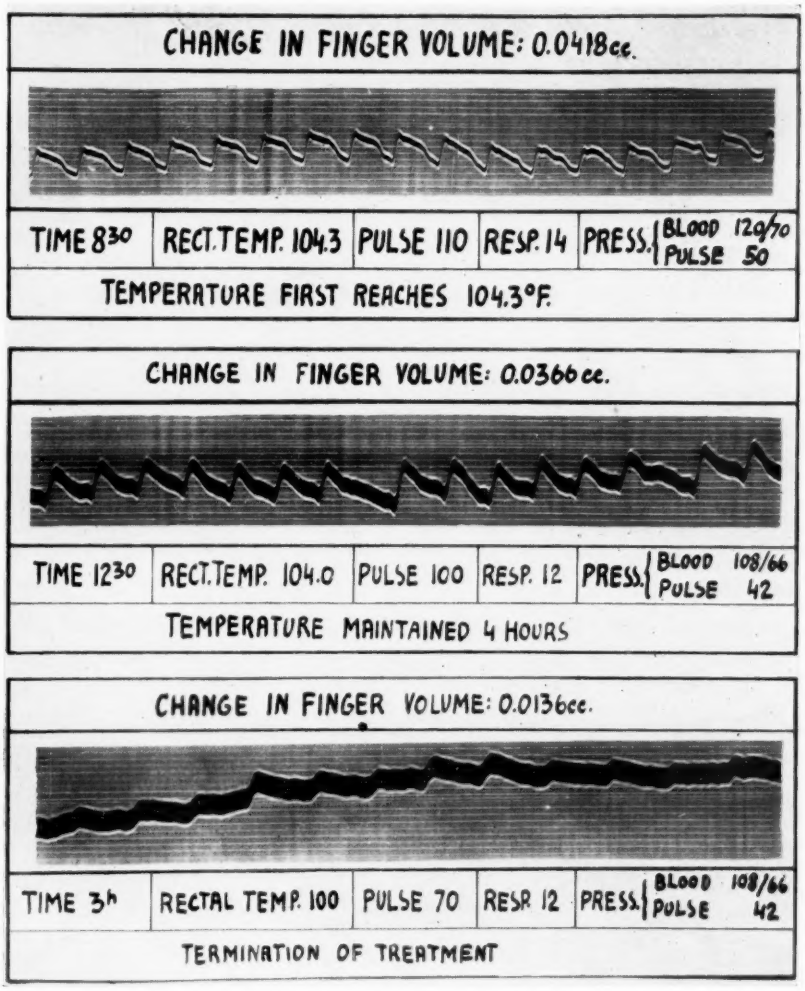

Fig. 1*B*.

Patient H. G. during his sixth and last treatment developed marked cyanosis as well as a twitching of the fingers of both hands. The heart tones were distant. The blood pH was 7.62. This patient's condition undoubtedly could have been relieved by the administration of carbon dioxide inhalations. Ten patients not listed completed only one or two treatments for various causes. One such patient after the second treatment developed a typical papular type of rheumatic rash, and treatment was, therefore, discontinued.

DISCUSSION AND SUMMARY

That none of our patients was "cured" has neither surprised nor dismayed us.

The current report[14] of the American Rheumatic Commission recently published by Hench and his collaborators shows only too well how far we are from the solution of the problems of arthritis. It indicates that there is far from unanimity among recognized authorities as to a standard classification of the disease in its various manifestations. The multiplicity, as well as the variety of the therapeutic procedures used for its control, certainly shows that the fundamental concepts of the disease are as yet little understood. Hench[10] states that fever therapy may benefit arthritis by (1) direct bacteriolytic or bacteriostatic effects due to an influence of heat itself on bacteria; (2) a local effect from vasodilatation providing an augmented blood supply to inflamed tissues; (3) increasing indirectly the formation or mobilization of immune bodies; (4) a general effect from the heightened metabolism incident to fever. Whatever may be the factor that accounts for the benefit of fever therapy in arthritis, Hench is certain that selection of the patient is of prime importance. We select our cases from the atrophic group alone, preferably those who have not had their arthritis longer than two years. Those whose arthritis is well advanced and arrested can hope for only slight improvement, such as gain in weight, less pain and stiffness, less fatigue, but very little improvement in joint function. Unquestionably no one yet can claim to have solved the problem of the correct temperatures to be employed, the duration of the fever or its frequency of application, nor have the possibilities of fever therapy used in conjunction with other procedures been fully explored.

Our patients were all referred to our clinic from outside sources for treatment and in most cases long after other forms of therapy had failed to give relief.

In this study it was our aim to evaluate further the results of the short fever curve maintained at 104° F. for four hours. These additional patients tend to confirm our previous impression[2] that improvement of the patient is greater and of longer duration when the fever is maintained for eight hours. In this series we also gave a minimum of six treatments, but undoubtedly this is far too few. It may well be that such a series of treatments repeated once or twice a year might produce much better results.

The plethysmographic records would seem to indicate that a rectal temperature exceeding 103° F. to 104° F. is neither necessary nor desirable. The maximum changes in the peripheral circulation apparently occur at this temperature level, and if increased circulation is the therapeutic objective, then these temperatures should not be exceeded. High temperatures are contraindicated for this type of patient because many of them give evidence of a vasomotor instability, and have a low threshold for exhaustion. Furthermore, it is our opinion that they should not be subjected to high environmental temperatures. It can be seen from Table II *D* that there is a very significant drop in blood pressure as a result of the fever treatment. This may be due to a low-

ered peripheral resistance, caused by the marked vasodilatation which is present, and to a lowered blood volume level.

Investigators vary markedly in their reports as to what constitutes the normal blood levels of ascorbic acid either in health or in disease. Abt and Farmer[15] consider blood plasma values below 0.7 mg. per 100 c.c. to be suboptimal. Daum, Boyd, and Paul[16] state that the normal ascorbic acid content of the blood ranges between 0.7 and 2.0 mg. per cent. The assumption has been made that fever therapy increases vitamin C requirement, but direct experimental proof is lacking. While the average ascorbic acid concentration of the blood for the group was 0.62 mg. before treatment, the range varied from 0.28 to 0.72. Many values found in these patients are considered to be very low normals. Thirty-three of the 77 determinations before treatment showed a level below 0.5 mg. This is of interest in light of Rhinehart's investigations. He found that subacute or chronic vitamin C deficiencies in guinea pigs produced an arthropathy with manifold similarities to rheumatoid arthritis, and that superimposed infection accelerated or accentuated the pathologic processes. Infection in the presence of vitamin C nutrition, he noted, failed to produce arthritis.

TABLE III

DATA OF BLOOD PROTEIN ANALYSIS

(26 Determinations)

	RANGE		AVERAGE
	MAXIMUM	MINIMUM	
Total blood proteins	9.2	6.02	7.37
Albumin	7.0	3.73	4.76
Globulin	4.7	1.02	2.53
Fibrinogen	0.82	0.17	0.42
A./G. ratio	5.2	0.84	1.9

It is apparent that artificial fever produces a significant elevation of the blood pH. The average pH value of 7.55 found at a temperature of 104° F. indicates a state of uncompensated alkalosis. The loss of carbon dioxide by the body through the lungs and sweat during the fever is undoubtedly of prime importance in increasing the alkalinity of the blood. The skin is a path of carbon dioxide loss of some importance. Koehler[17] is of the opinion there is a direct correlation between cyanosis and certain types of alkalosis. Mild cyanosis is not an uncommon occurrence in fever therapy. Koehler believes that fever alkalosis is due to increased lung ventilation and the rapid elimination of carbon dioxide from the blood, thus causing a carbon dioxide deficit, which in turn results in the passage of sodium ions into the tissue fluids and partially into the urine. Landis and collaborators[18] do not believe that hyperventilation is the sole cause of the pH change, but is dependent on several factors, such as the kidneys, the degree of sweating, and lactic acid formation.

Bischoff, Ullmann, Hill, and Long[19] do not agree with Koehler that an anoxemia exists in the presence of a fever alkalosis. As a result of the lowered carbon dioxide tension in the blood and increased pH, the stability of the oxyhemoglobin increases. If the circulation and the metabolism did not increase at the same time, the question would be quite simple. With an increase

in circulation, however, the tissues are exposed to more blood per unit of time so that the effect of the hemoglobin stability might be offset if the demand for more oxygen due to increased metabolism were not too great. It would seem then that instead of supplying the very distressed patient with oxygen as has been the tendency during the past few years, that it might be far more effective to give 8.5 per cent of carbon dioxide or even have the patient rebreathe air from a rubber bag. The unusual symptoms described for four patients are those resulting from hyperventilation of varying degree. Recently, Hinshaw and Boothby[20] have described this syndrome. Recently, also, we have undertaken some rebreathing experiments using a Boothby-Lovelace-Bulbulian face mask and as a result it is our opinion that had rebreathing been instituted during the period of hyperventilation all these patients would have completed treatment successfully.

Several investigations have been made chiefly on animals, a few on human beings, but none under comparable conditions, on the effect of fever on hemoconcentration. Observed blood changes[21] have frequently been explained as due to a concentration phenomena. According to Howell,[22] the specific gravity of human blood may vary from 1.041 to 1.067, the average being 1.055. All of our determinations (Table II) came within this range. Gibson and Kopp[23] state that one of the major physiologic effects of artificial fever induced by physical means is a diminution in circulating blood volume. They state that the degree of reduction in plasma volume is determined by the difference in rate of outflow by skin, lungs, and kidneys, and of effective absorption of fluids administered. If insufficient fluids are given the tissues, fluids of the body are drawn upon for the maintenance of plasma volume with resultant dehydration. Undoubtedly, the blood density will be dependent upon the balance of fluid loss and fluid gain, and one might readily criticize our work in that we did not control these variables. As previously stated, we gave fluids both in regard to quantity and rate, according to our judgment of the patient's need. We were interested, primarily, in ascertaining changes in hemoconcentration under the conditions of our therapeutic regime. In the thirty determinations of the specific gravity of the blood no significant changes were found. Apparently hemoconcentration is not the mechanism involved to account for the changes occurring in the blood cell count when fluids are administered during the course of treatment.

Our analysis of the blood serum proteins does not support the work of Davis.[24] Davis studied the blood plasma of nine patients with rheumatoid arthritis and found an increase of the globulin and fibrinogen as well as a lowered albumin of the blood plasma. The average albumin:globulin ratio of his nine patients was 0.9, while ours was 1.9. Two of our patients showed a reversal of the usual ratio (0.84 and 0.85, respectively). In both instances the reversal was due to an increase of the globulin fraction. Block[25] seems to believe that the albumin:globulin ratio is shrouded in a considerable degree of empiricism. Peters[26] states that studies of protein disturbances without simultaneous data on salt and fluid balance are valueless.

The average values for the total serum proteins, albumin, globulin, and fibrinogen of our patients conformed very well to the average normal values.

CONCLUSIONS

1. Twenty-seven patients suffering from chronic infectious arthritis were submitted to a fever of 104° F., maintained for four hours, and induced by electromagnetic induction.

2. Various physiologic observations, such as blood pH, ascorbic acid concentration of the blood, the specific gravity of blood, finger volume changes, total and fractional blood proteins, and blood pressures are reported.

3. Twenty-two per cent of the patients were markedly improved; 22 per cent moderately improved; 33 per cent slightly improved; and 22 per cent unimproved.

4. This form of therapy is not specific for chronic infectious arthritis but should be used in properly selected patients.

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THE AMOUNT OF PLASMA AND WHOLE BLOOD IN THE LUNGS OF DOGS*

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FEW previous studies concerning the amount of blood in the lungs are available. Stewart[1] calculated the quantity of blood in the lungs in dogs from the cardiac output per minute and the pulmonary circulation time, using the formula $V = Q \frac{60}{T}$. He expressed it as per cent of the total blood volume (estimated to be one-thirteenth of the body weight). His figures ranged from 11 to 26 per cent, and averaged 17 per cent. Kuno's[2] figures of 8.8 per cent to 19.44 per cent of total blood volume (estimated at one-fourteenth of body weight) in both lungs were obtained by colorimetric determination of the blood content of lungs from heart-lung preparations. Blumgart and Weiss[3] determined cardiac output and net pulmonary circulation time in human beings and, using Stewart's formula, calculated that the lungs contain 10 to 41 per cent of the total blood volume (estimated to be one-thirteenth of the body weight), the average being 21 per cent. Bruns[4] found that 6.3 per cent of the total blood volume in rabbits was in the lungs. Drinker, Churchill, and Ferry[5] tabulated the results of some previous investigators[6-9] and found, in three series of experiments, that in the rabbit the lungs contained 1/10.7 to 1/12.5 of the total blood volume at the end of inspiration and 1/15.3 to 1/17 of the total blood volume at the end of expiration. We have attempted to reinvestigate the question by a direct method of measuring the amount of plasma and (by calculation) whole blood in a single isolated lung.

PROCEDURE AND METHOD

Large normal mongrel dogs were used. Anesthesia was induced by the intravenous administration of pentobarbital (nembutal) in doses of 32.5 mg. per kilogram of body weight. After intubation of the trachea the chest was opened through the left fourth intercostal space. Adequate pulmonary ventilation was maintained by means of an Erlanger respirator. The pericardium was opened. The left branch of the pulmonary artery and the three left pulmonary veins were isolated and encircled separately by pieces of No. 1 chromic catgut, which were brought out through glass tubes, so that tension on the catgut closed the lumina of the encircled vessels. These tubes were bent in such a way as to conform to a space between the heart and the left lung, so that neither chambers

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of the heart nor portions of the left lung would be collapsed by mechanical interference. The glass tubes protruded from the chest wall, the layers of which were closed tightly about the tubes. Just before completing the closure of the wound, the lungs were fully inflated. To prevent pneumothorax, the ends of the glass tubes which protruded from the chest wall were sealed with bone wax through which the catgut ligatures passed. A moderately stout rubber band was then fastened to each catgut ligature. In Experiment 1 the intratracheal tube was removed, and spontaneous respiration was established. In Experiments 2 and 3 intermittent insufflation was continued throughout the remainder of the experimental period. After allowing several minutes for stabilization of the

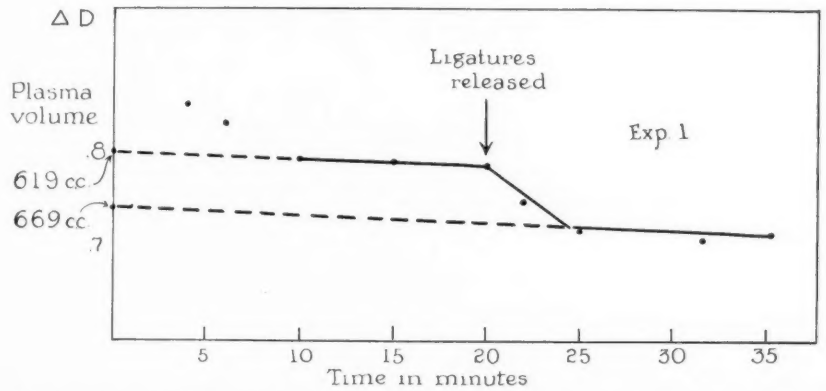

Fig. 1.—The concentration of dye in the circulating plasma is charted in relation to the intervals following (1) a single injection of dye, and (2) release of the ligatures. By extrapolation of the slopes thus obtained the circulating plasma volume before and after the release of the ligatures was determined. The difference represents the amount of plasma contained in the left lung.

circulation, the left lung was isolated from the general circulation by simultaneous traction on the four rubber bands. The plasma volume in the remainder of the circulatory system was then determined by the dye method of Gibson and Evans.[10] After twenty-one, twenty-one, and twenty-two minutes, respectively, in the three experiments, the ligatures were released, allowing the undyed plasma trapped in the lung to re-enter the general circulation. Blood samples for total plasma volume determinations were taken at intervals during the following twenty minutes.

The volume of plasma in the left lung was calculated as follows (see Fig. 1): The amount of circulating plasma was determined on the basis of the slope of the disappearance of the dye from the blood stream with the ligatures closing the lumina of the vessels of the left lung. On release of the ligatures the undyed plasma contained in the left lung further diluted the remainder of the plasma, and the total plasma volume was determined from the new disappearance slope (extrapolated to the time of the dye injection) obtained from the dye concentration of the blood samples taken after mixing was complete. The difference between the two volumes was taken to represent the amount of plasma contained in the left lung while it was isolated from the general circulation. Blood for hematocrit determinations was taken during both phases of the experiment.

TABLE I

CIRCULATING PLASMA VOLUME AND AVERAGE HEMATOCRIT BEFORE AND AFTER RELEASE OF THE LIGATURES; CALCULATED VOLUME OF PLASMA TRAPPED IN THE LEFT LUNG AND PERCENTAGE OF TOTAL PLASMA VOLUME IN THE LEFT LUNG AND IN BOTH LUNGS

	PLASMA VOLUME (C.C.)	AVERAGE HEMATOCRIT	PLASMA VOLUME IN LEFT LUNG (%)	PLASMA IN BOTH LUNGS (%)	REMARKS
Exp. 1, 19 kg.					
Ligatures closed	619	50.5			Animal breathing spontaneously
Ligatures open	669	50.25			
Volume of plasma in left lung	50		7.4	18.5	No pneumothorax
Exp. 2, 27 kg.					
Ligatures closed	910	58.4			Intermittent inflation
Ligatures open	962	59.7			Pneumothorax developed
Volume of plasma in left lung	52		5.4	13.5	
Exp. 3, 27 kg.					
Ligatures closed	920	57.2			Intermittent inflation
Ligatures open	995	60.1			No pneumothorax
Volume of plasma in left lung	75		7.5	18.7	

RESULTS

The results are shown in Table I. The left lung contained 7.4, 5.4, and 7.5 per cent of the plasma volume, respectively, in these three experiments. It has been shown that in dogs, the quantities of blood in the right and left lungs are in the proportion of 3:2.[2, 4] On this basis, the portion of the plasma volume in both lungs in these three animals was estimated to be 18.5, 13.5, and 18.7 per cent. The results in Experiments 1 and 3 are in close agreement, but the lower value in Experiment 2 may have been due to a pneumothorax of undetermined extent which was detected at the end of the experiment, as it has been shown that the quantity of blood in a lung decreases with collapse of lung tissue.[4, 11] Hematocrit values are shown in relation to closure and release of the ligatures in Fig. 2. There was little change throughout the procedure in Experiment 1, but in the other experiments there was a marked tendency for the hematocrit to rise for some time after the release of the ligatures. The possible significance of these findings is considered below.

DISCUSSION

The validity of the results obtained depends upon several factors affecting the accuracy of the method of plasma volume determination employed and upon the physiologic effects of the experimental procedures upon the circulation.

The accuracy of the plasma volume determinations by dye methods depends upon the complete mixing of the dye and plasma, and this, in turn, depends upon maintaining an adequate circulation as to both volume and speed. It has been demonstrated that under similar experimental conditions the sudden occlusion of one branch of the pulmonary artery does not cause a significant change in the cardiac output.[12] Thus, the same amount of blood goes through one lung as formerly went through both lungs, so that the circulation time is

decreased. Mixing of the dye and plasma was, therefore, thought to be satisfactory. The reliability of the so-called "indirect" method of determining changes in plasma volume with a single dye injection depends upon (1) the constancy of the intrinsic color (or optical density at a given wave length) of the plasma itself, and (2) the accuracy with which sudden changes in plasma volume are reflected by the differences in the disappearance slopes obtained. In the former instance, if there is marked blood concentration or dilution due to shifts in protein and pigment-free fluid, the optical density of the serum itself

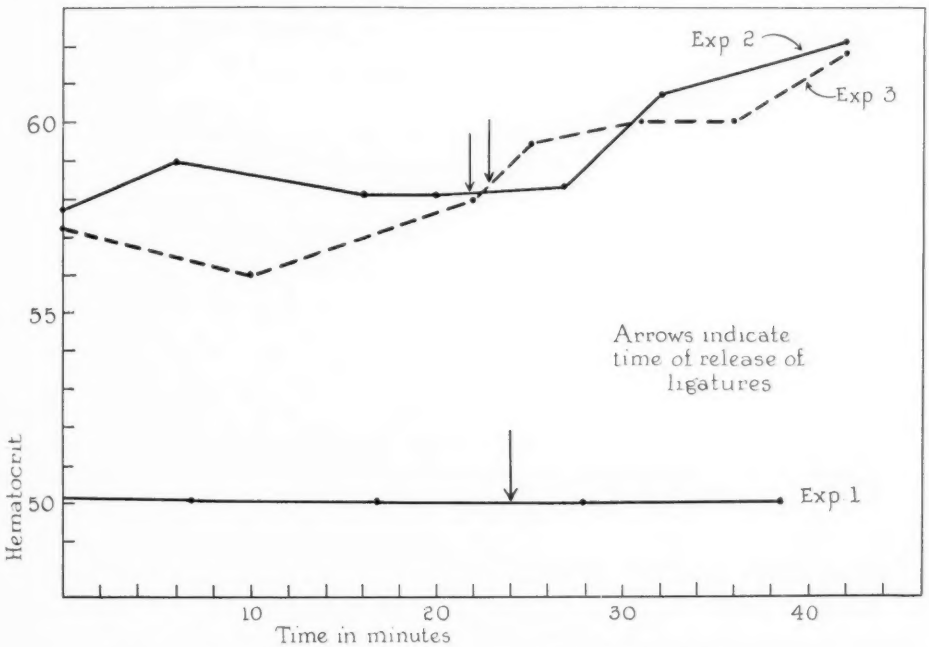

Fig. 2.—The hematocrit values in relation to the release of the ligatures.

will increase or decrease, and the net optical density, presumably due solely to the dye present, will give a distorted value. The magnitude of this error varies inversely with the concentration of dye used. Since the density value of dye in these experiments was from ten to twenty times that of dye-free serum, this source of error can be disregarded. There is little doubt that the extrapolation of a disappearance slope of the dye affords the most accurate dilution value upon which to determine the plasma volume. But if during such a control period, any great quantity of the dye disappears from the circulation, then the volume calculation based upon a second slope obtained after a sudden dilution, based on the same colorimetric constant for the original amount of dye injected, will be in error. In these experiments, not over 2 per cent of the dye left the blood stream during the time the initial slope was being obtained. Also, not over 10 c.c. (or about 1 per cent of average plasma volume) was withdrawn in sampling. Therefore, this objection may be dismissed as insignificant.

Far more serious criticisms may arise on the basis of the physiologic changes produced by the experimental procedure itself. It has been demonstrated that

no communication exists between the bronchial and pulmonary arteries, but there is a communication between bronchial arteries and pulmonary veins.[13] This communication is by means of capillaries, and the differences in size between the bronchial and pulmonary vessels do not suggest the possibility in these experiments of any great degree of mixture between dye-stained blood in the systemic circulation and dye-free blood in the pulmonary vessels of the left lung. However, such a communication might permit a small quantity of whole blood to enter the pulmonary circulation from the systemic circulation, but this quantity of blood would be added to that already isolated in the lung, there being no normal avenue by which blood may escape when once it is trapped in a lung by occlusion of the pulmonary veins. The disappearance of dye from the circulating blood stream through the bronchial arteries would not affect the determination of circulating plasma volume, because both dye and plasma would leave the systemic circulation in such proportions as to be without effect upon the concentration of dye in the systemic circulation. Similarly, when the tourniquets were released, the dilution which followed would be due to the original amount of dye-free plasma trapped in the lung.

Some anoxia may have occurred in the isolated lung, and the resultant increased permeability of the parenchymal capillaries may have permitted an escape of plasma, or of fluid poor in protein (and hence, dye) from the vascular bed. The rise in hematocrit in Experiments 2 and 3 (Fig. 2), after the release of the ligatures, suggests that this may have occurred. However, if the rise in hematocrit of the peripheral blood were due entirely to its mixture with the blood trapped in the left lung, the hemoconcentration should have been limited to the period in which dilution of dye occurred and should not have persisted thereafter (Fig. 1).

If stagnation of blood in capillaries in the dependent portions of the left lung occurred during the period in which it was isolated by the ligatures, it is possible that all the blood trapped in the lung was not carried out when the circulation was re-established. This possibility cannot be ruled out, but in that event the values for the amount of plasma contained in the lung would be too low.

We believe these results represent with reasonable accuracy the amount of plasma contained in the lungs of the dog in the resting state. No attempt has been made to determine the total blood volume in the lung from the change in plasma volume and the corresponding hematocrit values. It is not known definitely whether the ratio of plasma to cells is the same throughout the circulation, but there is increasing evidence that the corpuscular concentration is much less in capillaries than in large central or peripheral vessels, and that total blood volume calculated from the plasma volume and the hematocrit of blood from a large peripheral vessel are too high. It seems probable, however, that the hematocrit values of blood from the jugular vein (source of blood samples for hematocrit here) should not differ greatly from that in the pulmonary artery or veins, and it seems fair to say that the percentage of total blood volume in the lung is roughly equivalent to the percentage of plasma volume in the lung.

The results of this short series of experiments would not in themselves be of great significance, but there is close agreement between two of the determinations (Experiments 1 and 3), and it is believed that the presence of a pneumothorax accounts for the difference in the third (Experiment 2). The average value (17 per cent) is higher than that of Plumier (10 per cent), but compares favorably with that of Stewart (17 per cent). Our results in animals cannot be assumed to apply to human beings, yet it is interesting to note that Blumgart and Weiss[3] found that in man an average of 21 per cent of the total blood volume was contained in both lungs.

SUMMARY

The quantity of blood contained in the left lung was trapped by the simultaneous occlusion of the vessels to that lung while both lungs were fully expanded. From determinations of the circulating plasma volume, during this period and after the release of the occlusion, the quantity of plasma trapped in the left lung was measured.

In three experiments, 7.4, 5.4, and 7.5 per cent (average 6.8 per cent) of the plasma volume was contained in the left lung. On the basis that, in the dog, the right lung contains one and one-half times as much blood as the left lung, the quantity of plasma in both lungs averaged 17 per cent. For reasons explained, a similar percentage of total blood volume may be assumed to be contained in the lungs.

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IODINE ALLERGY*

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THE widespread use of iodine and its salts in modern diagnostic and therapeutic techniques makes the finding of unusual reactions to these drugs extremely likely. Although the use of tincture of iodine as an antiseptic has fallen off in recent years, the use of the iodides, organic and inorganic, has increased because of their value in diagnosis. Prolonged intake of iodine or its salts gives rise to cutaneous eruptions more often than does the continued intake of any other single drug. However, these cases have to be distinguished from others that react in an unusual way to only a minute quantity of iodine. The latter patients are said to exhibit iodine hypersensitivity or allergy.

A patch test made with a drop of tincture of iodine, and read twenty-four hours later revealed marked redness and swelling over the site in all the hypersensitive patients. If this can be shown to be a general finding in other cases, it can be used as a diagnostic test. Other nonsensitive persons tested in this way did not show this reaction.

CASE REPORTS

CASE 1.—Reaction following the drinking of a saline laxative containing a minute quantity of iodine.

G. B., an 18-year-old girl, took a teaspoonful of an imported Italian salt, which was found to contain a very small quantity of iodine. Twelve hours later her eyes became red, her face started to swell, and she developed a pin-point, reddened eruption over her entire body, closely resembling that of scarlet fever. Her temperature was normal. An iodine patch test was markedly positive, and the qualitative examination of the urine for iodine and iodides was negative. The white blood cell count and the differential were within normal limits. The rash cleared rapidly after the administration of 10 grain capsules of sodium chloride every two hours for six doses. This patient had no previous history of sensitivity to iodine in any of its forms. She had used the salt repeatedly for years, but only occasionally during the preceding year.

CASE 2.—Reaction following the taking of a commonly used throat lozenge containing anesthesin and calcium iodide.

R. F., an 18-year-old girl, took a lozenge for relief of sore throat. Twelve hours later she developed a slight fever, swollen neck glands, slight edema of the face, and a generalized, reddened pin-point cutaneous eruption resembling that of scarlet fever. Her throat was slightly red; differentiation from scarlet fever in this case was difficult. However, the Schultz-Carlton test was negative, and an iodine patch test was markedly positive. The white blood cell count was 9,200, with 84 per cent polymorphonuclear cells. The qualitative

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examination of the urine was negative for iodine and iodides. The symptoms cleared up in twenty-four hours after the taking of several fruit drinks containing large quantities of ordinary table salt, and without other medication.

After careful questioning this girl gave a history of having had a similar eruption many years before, which was diagnosed as scarlet fever. She had no sore throat at the time. She recollected having bruised her knee and applying tincture of iodine. The bruise turned a livid red, and a generalized rash appeared approximately twelve hours later.

Case 3.—Cutaneous reaction following the insertion of a small piece of iodoform gauze after removal of a lipoma of the neck.

P. S., a 45-year-old male, had a lipoma removed under general anesthesia. A small amount of iodoform gauze was placed in the wound as a drain. Several hours later his temperature rose to 102° F. He showed lassitude, refused to take food, and had swelling of the face, eyes, and hands. The white blood cell count was 8,800, with 82 per cent polymorphonuclear cells. An iodine patch test was positive. The iodoform pack was removed. The patient's symptoms cleared within twenty-four hours following administration of 5 grains of sodium chloride every four hours.

Case 4.—Thrombocytopenic purpura following the application of iodine percutaneously.

A. G., a 55-year-old Italian male, developed pains in his right shoulder and left ankle. He applied a thick layer of tincture of iodine to both areas. Several hours later he developed purpuric spots all over his extremities, chest, and abdomen. There was marked swelling of both hands, as well as of the face, and especially over those areas where iodine had been painted. There was no palpable liver or spleen, and the temperature was normal. The platelet count was 80,000. The bleeding time was fifteen minutes, coagulation time was five minutes, and clot retraction was still absent the following day. The white blood cell count was 8,200, with 78 per cent polymorphonuclear cells. There was a positive tourniquet test. An iodine patch test read the following day showed marked redness. On the assumption that this was a case of thrombocytopenic purpura, based on an allergic reaction to iodine, this man was given 5 grains of sodium chloride every four hours. The purpuric spots steadily cleared up, the redness in his face disappeared, and the swelling of both hands slowly receded. One week later the platelet count was 140,000, the bleeding and coagulation times were within normal limits, and there was good clot retraction in two and one-half hours.

About two weeks after the initial episode, he came to the office feeling very well and asked to have a Wassermann test done. An iodine swab was accidentally used to sterilize the patient's antecubital fossa preparatory to venipuncture. This was immediately swabbed with alcohol. Eight hours later the patient had a chill, swelling of the skin of the antecubital fossa, and a reappearance of purpuric rash. The platelets were again depressed to 70,000, the bleeding time was prolonged to ten minutes, the coagulation time to five minutes, and there was absence of clot retraction. These findings again cleared up after 5 grain sodium chloride capsules every four hours for three days. Within ten days the platelets had returned to 200,000, and the bleeding and coagulation times became normal. The Wassermann reaction was negative.

Case 5.—Cutaneous eruption following the application of tincture of iodine.

A. B., a 15-year-old girl, developed a fever of 102° F., redness and swelling of the hands and face following the application of tincture of iodine to a bruise on the skin. The bruise was surrounded by an erythematous, raised red area, resembling erysipeloid. Diagnosis was made after the patient gave a history of a previous similar experience three years

before. The iodine patch test was markedly positive. Urine determinations for iodine and iodides were negative. The symptoms cleared up promptly after several doses of sodium chloride in 5 grain capsules.

COMMENT

Considering the widespread use of iodine and iodides, the incidence of allergic reactions to these substances appears to be low. That they must be considered, however, is brought out by the many deaths reported in the literature. Hollander and Fetterman[1] reported the eleventh case of fatal allergic reaction in 1936. Since that time there have been at least seven more recorded instances. In our series we were fortunate in not having deaths, although some of the reactions were quite severe.

Three of our cases showed general reactions to cutaneously applied tincture of iodine. One developed a scarlatiniform eruption, another had a history of such an eruption many years before. The third patient developed a case of thrombocytopenic purpura, typical in all respects save enlargement of the spleen. The literature on hypersensitivity to the cutaneous application of iodine is covered in Seymour's article,[2] in which he adds a very instructive case. The patient was tuberculous, and iodine was applied prior to the institution of pneumothorax treatment. After several moderately severe reactions every time iodine was exhibited, a very severe reaction occurred which resulted in death. Before death the patient developed an exfoliative dermatitis.

The question of the use of iodoform packs following operation is a moot one. Cushny[3] stated that iodoform was almost useless as an antiseptic. He showed that microbes drawn from wounds under iodoform treatment were not retarded or weakened in their development. Indeed, he quoted work showing that the emigration of leucocytes from the blood vessels was hindered by the local action of iodoform. This would tend to retard healing. Iodoform slowly decomposes into iodine in the body, but the quantity so formed per unit time cannot be sufficient to sterilize the wound. It leads to a false sense of security, and when the question of sensitivity is taken into account, one wonders whether its use is justifiable.

The incidence of thrombocytopenic purpura following iodine allergy has not been so adequately discussed. Osler[4] described a case which commenced twenty-four hours after the taking of 10 drops of compound of tincture of iodine. Dennig[5] described three cases, all in elderly women with Basedow's disease. One followed the taking of potassium iodide in 6 drop doses daily for three weeks. Purpura developed. The platelets dropped to 20,000. Several weeks later the platelets increased and the patient finally recovered. Another of his cases is confused because of the coincident use of sedormid, together with tablets of an iodide compound. A third patient developed purpura following the consumption of sodium iodide for a short time. Both of these patients subsequently recovered.

There are several other cases with hemorrhagic symptoms in the literature, but unfortunately blood studies are lacking. Lobsenz[6] reported fatal iodine poisoning following the use of iodoform gauze drains. He attributed the severe

hemorrhagic symptoms that the patient developed to vitamin C deficiency, but this appeared to be an impression unsupported by laboratory tests. Rowell[7] reported a case showing hemorrhagic blebs on the extremities. The clotting time was between six and seven and one-half minutes. No figures were available on the platelet count.

All our cases showed local cutaneous sensitivity to tincture of iodine on patch test. This consisted of marked reddening and itching, perceptible in five to six hours. When observed at twenty-four hours, the erythematous area usually had extended slightly and was edematous. Since this reaction was invariable, irrespective of the method of intake of iodine or its salts in these patients, it was used as a method of detecting sensitivity, and called the iodine patch test. Jacobs[8] found essentially the same result in his case, although he used other iodine products and iodine ointment as well. However, in an unduly sensitive person, even one drop of tincture of iodine might conceivably cause a marked reaction. Hence it might be better to wipe off the iodine with some alcohol after a minute. Since our patients did not have marked reaction to one drop, we have not as yet used this precaution.

In our patients, the dose of iodine taken into the body was so small that qualitative tests for iodine and iodides in the urine were negative. Sodium chloride in 5 or 10 grain doses appeared to ameliorate the symptoms. This appears to be the usual method of treatment. One author[9] used sodium chloride intravenously. There are several reports of the use of epinephrine.[10] If the mechanism is indeed one of allergy, this latter treatment seems more logical. The rationale of the sodium chloride treatment is that the chlorine ion displaces the weaker iodine from the iodide. In very severe cases the use of adrenal cortex hormone, because of its action on the sodium chloride in the blood, would seem to be indicated along with the other means at our disposal.

The mechanism of iodine allergy is unknown. The Wolf-Eisner theory of the combination of iodine with a protein in the body to produce the antigen is not substantiated by Jacobs. This theory, although intriguing, still awaits proof.

The patient who has iodine allergy must be told of his condition. Failure to do so may place his life in jeopardy at a later date. The physician who plans to use iodine or one of the iodides in diagnosis or treatment should ask the patient whether he has any iodine sensitivity. The ideal procedure would be to patch test every prospective user of these compounds. However, this is not often possible because of the time element involved.

SUMMARY

1. Five cases of hypersensitivity to iodine and its salts are described.

2. A patch test for the detection of this sensitivity is described, and was present in all five patients.

3. Several types of cutaneous eruption followed the use of iodine or its salts in these patients, appearing in from eight to twelve hours. Scarlatiniform eruptions developed in two, and a purpuric rash in another.

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A BACTERIOLOGIC CHECK ON RESIDUAL DENTAL INFECTIONS*

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THAT the mere removal of infected teeth does not always suffice to eradicate dental infection has been taught by several authors. Martin H. Fischer's recent book, *Death and Dentistry*,[1] clearly re-emphasizes the problem. Removal of infected teeth alone "cures" the constitutionally afflicted in only some one-third of the cases, and "improves" the state of another third. There is abundant clinical and roentgenologic evidence that when teeth are extracted in the ordinary fashion, residual infection in the surrounding alveolar tissues may be left in place. Root fragments and bone rarefactions are conceded to be a source of systemic infection by most oral pathologists, but we would here emphasize the still larger importance of the infection remaining in the alveolar structures when such grosser evidences of pathology are nonexistent.

We have added to clinical and x-ray examination a bacteriologic study of cultures made from the alveolar structures. X-ray photographs of the full mouth were taken of patients presenting constitutional signs of chronic infection, after other possible sources for systemic invasion had been ruled out. These films showed an irregularity and furriness of the alveolar processes assumed by us to be caused by residual infection. The matter is illustrated by Figs. 1 and 2. In all the patients observed the teeth had been extracted at least five years previous to this study.

In a first series of edentulous patients who showed signs of residual infection by x-ray, as described above, a general anesthetic (pentothal sodium) was administered intravenously. When it had taken effect, the jaws were held open and the alveolar areas to be examined were scrubbed with soap and water.

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This was followed by a topical application of tincture of iodine, then alcohol, and finally, tincture of merthiolate. Swab cultures made from the surfaces thus prepared were negative in 8 of 10 patients studied, the remaining two patients showing organisms in no way related to the organisms cultivated from the

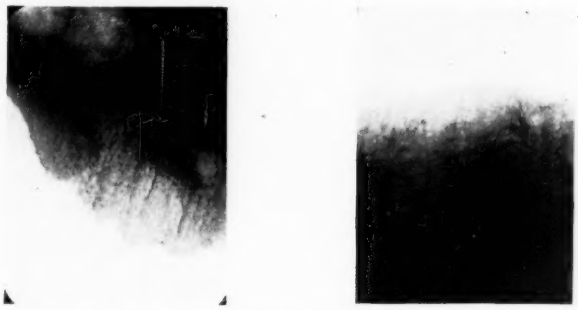

Fig. 1.—Note furriness and irregularity of the alveolar processes of a patient from whom all cultures made by puncture or grinding yielded a streptococcus.

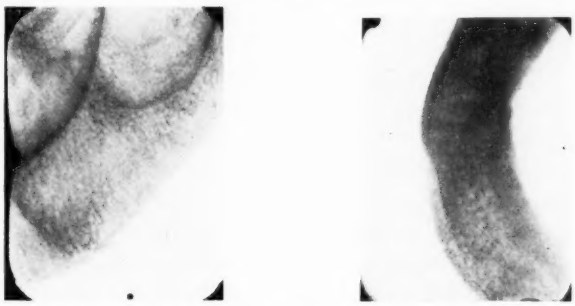

Fig. 2.—Note smooth contour of mandibular surfaces in comparison with Fig. 1. Stab and incision cultures from this patient proved negative.

underlying structures. After this sterilization of the surface, 1 c.c. of sterile saline solution was injected into the alveolar processes, allowed to remain there a few moments, and then withdrawn without removal of the needle in the interim. We could thus recover approximately 0.25 c.c. This was squirted into sterile tryptose broth. Twenty-four hours later subcultures were made. In the 10 patients showing no more than the alveolar furriness described above, organisms were recovered from all. In a control series of 6 patients presenting smooth alveolar ridges, cultures taken in a like manner always proved negative.

In a second series of 16 patients incision was made, and the alveolar bone was exposed after proper precedent cleansing. This time cultures were made of the bone grindings. Growth of organisms was obtained in every instance. The grinding was done with a sterile burr. Check of the organisms recovered at times from the buccal surfaces against those recovered from the alveolar processes showed them to be wholly nonrelated.

The foregoing indicates that the removal of teeth is not synonymous with the eradication of a locus of infection, that mere irregularity and furriness in contours of after-remaining alveolar processes as revealed by the x-ray are evidence of the continued existence of such a locus.

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THE SIGNIFICANCE OF ELECTROCARDIOGRAPHIC CHANGES IN MALIGNANT HYPERTENSION*

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INTRODUCTION

THE changes that occur in the electrocardiogram of patients who have essential hypertension have been studied extensively. The exact nature of these changes is not fully understood, and there is much disagreement as to their significance.

In an attempt to clarify the controversy, a study of the fatal cases of malignant hypertension recognized at the Medical College of Virginia over a seven-year period was undertaken. Care was exercised in the selection of cases in order to eliminate any extraneous factors, such as valvular defects or coronary thrombosis, which might be present. Thirty-seven cases were available for study, comprising 54 electrocardiograms and 16 autopsy examinations.

The malignant phase of hypertension was chosen for several reasons. The course of the disease is very rapid, telescoping into a few months or years the changes that occur in essential hypertension over many years. The short time interval reduces the possibility of accidental occurrences which might alter the electrocardiogram. The disease usually terminates before senile degenerative changes have begun to appear. In practically all of these cases the patient died of uremia. Heart failure was not clinically present to complicate the electrocardiographic picture. It may be stated categorically that the changes seen in these electrocardiograms and observed at the autopsy table arose because of the presence of malignant hypertension.

THEORETICAL CONSIDERATIONS

Many authors have noted changes in the electrocardiogram which occur when there is marked left ventricular preponderance. Master[1] states that there is a definite relationship between the appearance of these changes and the size of the heart, and implies that the size of the heart is the sole factor involved. Barnes and Whitten[2] suggest that the size of the heart may be responsible for some of the changes, but not all, and that certain changes in the T wave are indicative of "left ventricular strain" or myocardial damage. In this study an attempt has been made to correlate post-mortem findings with the electrocardiographic changes.

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Willius[3] was among the first to provide indication that in hypertensive heart disease there is a change in the T wave. He found that in cases exhibiting T-wave negativity in Lead I, or in Leads I and II, the predominant disease was hypertension. This was not true of other combinations of T-wave changes. Barnes and Whitten[2] found that where lesions increased the work of the left ventricle, inversions of the T wave were often present in Lead I, or in Leads I and II; similarly, right ventricular preponderance produced T-wave inversion in Leads II and III, although the correlation was not as close as in left ventricular preponderance. They found that in nearly two-thirds of the patients with simultaneous inversions of the T wave in all leads there was no evidence of ventricular preponderance. From this they concluded that the factors producing ventricular preponderance were not the same as those producing T-wave inversions. They hypothesized further that the changes were evidence of ventricular strain, and were not dependent on the size of the heart.

Master[4] holds a different opinion; he contends that the changes occurring in arterial hypertension are due solely to the increase in the size of the heart over a rather long period of time. His study consists largely of correlations between the electrocardiograms of patients with essential hypertension and the size of the heart as determined by the cardio-thoracic ratio in the teleroentgenogram. He states[1] that, as enlargement of the left ventricle begins, left axis deviation develops in the electrocardiogram. With further increase in the size of the left ventricle, T-wave inversion in the first lead occurs, followed by inversion of the T wave in the second lead. Roesler and co-workers[5] noted a steady increase in the percentage of cases with electrocardiographic final deflection changes as the radiologic examination showed an increase in the size of the heart, but they found that an absence of final deflection changes and of left axis deviation was compatible with a marked degree of cardiac enlargement. They state that some factor other than cardiac enlargement is necessary to produce these changes.

Odel[6] found structural changes in varying degrees of severity in the arterioles of the myocardium in a series of 35 cases of malignant hypertension. The presence of diffuse fibrosis in the myocardium in these cases added further evidence to the supposition that the electrocardiographic changes were due to myocardial damage. The electrocardiograms of these patients revealed depression of the S-T segment in Leads I or II in 59 per cent, and abnormal T waves in those leads in 47 per cent. Gouley[7] states that myocardiopathy is a common occurrence in uremia with severe hypertension, while Wood and White[8] attribute associated electrocardiographic changes to the toxic effect of uremia on the myocardium.

PRESENT STUDY

The present study may be divided into several parts. They consist of a consideration of the predominant trends in the electrocardiograms of 37 patients who died of malignant hypertension; the correlation of T-wave changes to myocardial damage as proved at autopsy; the relationship of cardiac weight to cardio-thoracic ratio; the relationship of myocardial damage to cardio-thoracic ratio; and the prognostic significance of the changes observed.

I. PREDOMINANT CHANGES IN THE ELECTROCARDIOGRAM

A detailed analysis of all important features of the electrocardiograms in the cases studied is shown in Table I. The total number of electrocardiograms showing the particular changes are noted under each heading. From this basic chart Table II is derived. It was felt that all abnormalities of the T wave should be considered, and we believe that the results justify this procedure. A total of 75.9 per cent of the electrocardiograms showed abnormalities of the T wave in Lead I and/or Lead II. Left axis deviation was present in 59.3 per cent, while depression of the S-T segment in Lead I and/or Lead II was found in 50 per cent.

TABLE I

FUNDAMENTAL DATA: ANALYSIS OF 54 ELECTROCARDIOGRAMS IN 37 PATIENTS

CHANGES	LEADS						
	I	II	III	I AND III	II AND III	I AND II	I, II, AND III
Notched P	0	3	1	1	4	0	1
Diphasic P	0	1	0	0	0	0	0
Negative P	2	0	3	0	0	0	0
High voltage P	0	2	2	0	2	0	0
High voltage R	8	3	0	0	0	2	2
High voltage QRS	1	2	0	0	0	0	5
Deep S	0	1	25	1	11	0	0
Q wave	8	0	0	0	0	0	0
Depressed S-T	9	7	0	0	3	11	0
Slurred QRS	2	3	10	5	2	0	3
Elevated S-T	0	0	9	0	0	0	1
Prolonged S-T	0	0	0	0	0	0	1
Convex S-T	5	1	1	0	1	0	0
Concave S-T	0	0	3	0	0	1	0
Depressed S-T, upright T	3	4	0	0	0	1	0
Depressed S-T, negative T	7	7	0	0	1	4	0
Elevated S-T, upright T	0	1	3	0	0	0	0
Elevated S-T, negative T	1	0	3	0	0	0	0
Low T	7	2	3	3	2	1	0
Flat T	2	3	0	1	1	0	0
Diphasic T	8	8	3	1	3	2	4
Negative T	5	1	2	1	1	2	1
Total T changes	22	14	8	6	7	5	5

Left axis deviation was present in 32, absent in 22.

TABLE II

RELATIVE INCIDENCE OF PREDOMINANT CHANGES IN THE ELECTROCARDIOGRAMS OF 37 PATIENTS WITH MALIGNANT HYPERTENSION (54 EKG)

	PER CENT
Changes (low, flat, diphasic, or negative) in T_1, T_2, or T_1 and T_2	75.9
Deep S_3, or S_2 and S_3	69.3
Left axis deviation	59.3
Depressed S-T_1, S-T_2, or S-T_1 and S-T_2	50.0
Deep S_3	46.3
Depressed S-T_1, S-T_2, or S-T_1 and S-T_2 and negative T_1, T_2, or T_1 and T_2	33.3
High voltage R_1, R_2, or R_1 and R_2	24.1
Elevated S-T_3	16.7
Depressed S-T_1, S-T_2, or S-T_1 and S-T_2 with upright T complexes in those leads	14.8
Q wave, Lead I	14.8

These results show that in malignant hypertension the variations in the T wave and the S-T segment must be considered a concomitant of the disease. When S-T depression is associated with abnormalities of the T waves, it means there is a more severe effect of the disease upon the heart than the T-wave changes alone would indicate.

II. ELECTROCARDIOGRAPHIC CHANGES AND MYOCARDIAL DAMAGE

In view of the controversy as to the presence or absence of myocardial damage in cases showing T-wave changes in the electrocardiogram when hypertension is present, we have analyzed the cases in our records which were autopsied. The high incidence of myocardial damage in cases showing T-wave changes in the electrocardiogram, and the absence of changes in cases where no damage was demonstrable, show that the changes indicate myocardial damage. This is demonstrated in Table III. A positive correlation is present in 68.75 per cent of cases.

TABLE III

CORRELATION OF T-WAVE CHANGES IN LEADS I AND/OR II WITH PRESENCE OR ABSENCE OF MYOCARDIAL DAMAGE ON POST MORTEM (16 CASES)

	CASES	PER CENT
Damage with EKG changes	7	43.75
Damage without EKG changes	2	12.50
EKG changes without damage	3	18.75
No damage and no EKG changes	4	25.00

A positive correlation is present in 68.75 per cent of the cases, and a negative correlation in 31.25 per cent. While these figures are derived from too small a series to prove the point beyond question, they confirm the impression that the T-wave changes in malignant hypertension are due to myocardial damage, and not to enlargement of the heart.

III. CARDIAC WEIGHT, MYOCARDIAL DAMAGE, AND CARDIO-THORACIC RATIO

Fourteen patients were available for analysis of the cardiac weight. Nine of these had a determination of the cardio-thoracic ratio before death. The mean cardiac weight was 586 Gm., and the mean cardio-thoracic ratio was 55 per cent. The deviations from the mean were considerable, the smallest heart weighing 400 Gm. and the largest 820 Gm., while the cardio-thoracic ratio ranged between 45 and 66 per cent. The interesting part of the analysis is the lack of correlation of the size of the heart to the cardio-thoracic ratio. There are many reasons for this, as shown in the footnote to Table IV.

We have analyzed our data in 20 cases in which the cardio-thoracic ratio was known. T-wave changes indicative of myocardial damage were present in 15 of these cases; the cardio-thoracic ratios in these cases ranged between 45 and 66 per cent, covering the entire range of our series. On the other hand, electrocardiograms showing no T-wave changes in five cases were also found to cover the entire range of cardio-thoracic ratios. The results are tabulated in Table V.

These findings suggest that there is no definite correlation of cardiac size with cardio-thoracic ratio, nor of electrocardiographic changes with cardio-thoracic ratio. The cardio-thoracic ratio is a totally unreliable guide in interpreting the changes in the electrocardiogram. It cannot be assumed that a high cardio-thoracic ratio means that the electrocardiographic final deflection changes are without significance.

TABLE IV

CORRELATION OF CARDIAC WEIGHT TO CARDIO-THORACIC RATIO

CASE NO.	CARDIAC WEIGHT (GM.)	C-T RATIO (%)	WEIGHT DEVIATION FROM MEAN	RATIO DEVIATION FROM MEAN
S4234F	400	52	-186	- 3
S13460F	470	45	-116	-10
S52132M	500	45	- 86	-10
S38192M	500	56	- 86	1
S42537F	530	--	- 56	---
S40224M	550	66	- 36	11
S13584M	580	60	- 6	5
S32122F	590	58	4	3
M51122F	600	55	14	0
M27562M	610	--	24	---
S4019M	650	--	64	---
S32128M	660	54	74	- 1
M45348M	750	--	164	---
S45189M	820	59	234	4

The mean cardiac weight is 586 Gm. The mean cardio-thoracic ratio, as determined by teleroentgenogram is 55 per cent. Statistical analysis of this very small series shows that, although there is a positive correlation, it is not greater than that which could occur by chance (coefficient of correlation is 0.435, standard error of coefficient of correlation is 0.354). Factors modifying the C-T ratio, such as degree of dilatation of the ventricles, the rotation of the heart in the body, and the anteroposterior diameter of the chest, render this an unreliable method of determining the true size of the heart.

TABLE V

RELATIONSHIP BETWEEN T-WAVE CHANGES INDICATIVE OF MYOCARDIAL DAMAGE AND THE CARDIO-THORACIC RATIO IN THE SAME PATIENTS

MYOCARDIAL DAMAGE	CARDIO-THORACIC RATIO PER CENT
No	45
Yes	45
Yes	51
Yes	51
No	52
Yes	52
Yes	53
Yes	54
Yes	55
Yes	55
Yes	55
No	56
No	58
Yes	59
Yes	60
Yes	60
Yes	61
Yes	64
Yes	66
No	66

As shown above, no correlation exists between the cardio-thoracic ratio and T-wave changes indicative of myocardial damage.

IV. THE PROGNOSTIC SIGNIFICANCE OF THE ELECTROCARDIOGRAM OF MALIGNANT HYPERTENSION

It is recognized that the prognosis of malignant hypertension is serious, death usually occurring a few months to a few years after the disease has been discovered. It is still impossible to predict for the individual patient whether or not he will have the greater life expectancy of one or two years.

In an attempt to provide a more exact prognosis, we studied the electrocardiograms of each patient who had more than one electrocardiogram taken before he died, with special regard for the changes in the S-T segment and

TABLE VI

CHANGES IN S-T SEGMENTS AND T WAVES IN REPEATED ELECTROCARDIOGRAMS ON 11 PATIENTS DYING FROM MALIGNANT HYPERTENSION

CASE NO.	DATE	TIME INTERVAL	S-T AND T CHANGES	DATE DIED	DAYS AFTER LAST EKG
M51122F	4/18/40		None	5/ 4/40	10
	4/25/40	7 days	Elevated $S\text{-}T_1$, $S\text{-}T_2$, $S\text{-}T_3$,* flat T_1, low T_2		
S79386M	3/21/40		Depressed $S\text{-}T_1$, $S\text{-}T_2$, diphasic T_1, T_2, T_3	5/31/40	43
	4/18/40	28 days	Depressed $S\text{-}T_1$, $S\text{-}T_2$, concave $S\text{-}T_3$, low T_1, T_3, diphasic T_2		
S82984F	8/27/40		Depressed $S\text{-}T_1$, $S\text{-}T_2$, low T_1, T_2	9/14/40	12
	9/ 2/40	5 days	Low T_2, negative T_3		
S52132M	5/13/38		Diphasic T_1	11/22/38	0
	9/ 2/38	4 months	Elevated $S\text{-}T_3$		
	11/22/38	2½ months	Low T_1, diphasic T_2, T_3		
M45348M	10/20/37		Depressed $S\text{-}T_2$, low T_1, T_3	11/ 4/37	13
	10/22/37	2 days	Diphasic T_1		
S32122F	1/ 2/36		Depressed $S\text{-}T_1$, negative T_1, diphasic T_2	1/18/36	6
	1/12/36	10 days	Low T_1, diphasic T_3		
S25555F	2/13/35		Depressed $S\text{-}T_2$, diphasic T_1, T_2, T_3	2/24/35	5
	2/19/35	7 days	Depressed $S\text{-}T_2$, $S\text{-}T_3$, low T_1		
S26791M	4/18/35		Depressed $S\text{-}T_1$, negative T_1, T_2, T_3	5/21/35	6
	4/29/35	11 days	Depressed $S\text{-}T_2$, $S\text{-}T_3$, low T_1, diphasic T_2, T_3		
	5/15/35	16 days	Depressed $S\text{-}T_1$, $S\text{-}T_2$, diphasic T_2, T_3, low T_1		
S24735F	12/26/34†		Depressed $S\text{-}T_1$, $S\text{-}T_2$, concave $S\text{-}T_1$, $S\text{-}T_2$, convex $S\text{-}T_3$, low T_1, flat T_2, T_3	1/10/35	2
	12/31/34	5 days	Depressed $S\text{-}T_1$, $S\text{-}T_2$, elevated $S\text{-}T_3$, flat T_1, T_3, diphasic T_2		
	1/ 3/35	3 days	Convex $S\text{-}T_1$, flat T_1, T_2, negative T_3		
	1/ 8/35	5 days	Depressed $S\text{-}T_1$, $S\text{-}T_2$, elevated $S\text{-}T_3$, negative T_1, diphasic T_2		
S19858M	4/25/34		Diphasic T_1, flat T_2	5/15/34	4
	5/ 3/34	8 days	Depressed $S\text{-}T_1$, $S\text{-}T_2$, convex $S\text{-}T_1$, diphasic T_1, T_2, T_3		
	5/11/34	8 days	Depressed $S\text{-}T_3$, diphasic T_1, T_3, flat T_2		
S13460F	5/14/34		Negative T_3	11/ 1/35	38
	9/24/35	16 months	None		

*Consistent with pericarditis.

†Digitalis effect is present in this electrocardiogram.

Eighty-one per cent of these patients showed changes in the S-T segment or T wave characteristic of malignant hypertension in at least one electrocardiogram. All of them died in uremia; nevertheless, the electrocardiographic changes have considerable prognostic significance. Malignant hypertension is a diffuse arteriolar disease. When the coronary arterioles have become involved to a degree sufficient to produce myocardial damage, renal failure is near.

T wave. Eleven cases were available, with 27 electrocardiograms. It was found that 81 per cent of these patients showed characteristic S-T or T changes in at least one electrocardiogram. This may be explained, since the single electrocardiogram in the other cases might not have been taken at the most opportune time to demonstrate the changes. As shown by Bellet and McMillan,[9] terminal uremic pericarditis often elevates the S-T segment in all leads. It is likely that in some of our cases it raised the depressed S-T segment to the isoelectric line. The study indicates that in a very large majority of cases, characteristic electrocardiographic changes can be demonstrated at some time during the patient's life.

There was no uniformity in time from the appearance of the characteristic electrocardiogram to the death of the patient. However, as shown in Table VI, the appearance of these changes meant that death was near. When the widespread vascular process had proceeded long enough to produce changes in the heart muscle sufficient to give the characteristic electrocardiographic picture, the kidneys were usually on the verge of complete failure. While the electrocardiogram cannot predict the exact life expectancy, its changes indicate a more serious prognosis for the immediate future than does the normal electrocardiogram with the same degree of hypertension.

TABLE VII

FREQUENCY OF DEATHS FROM MALIGNANT HYPERTENSION AT VARIOUS AGES, GROUPED AT FIVE-YEAR INTERVALS

AGE IN YEARS	NUMBER DYING
15-19	1
20-24	2
25-29	3
30-34	7
35-39	10
40-44	8
45-49	6

Table VII shows that the greatest mortality is after the third decade, with no deaths after 50 years of age. This indicates that the changes found in the electrocardiograms of these patients are due almost entirely to the malignant hypertension. The degenerative senile processes, which may alter the electrocardiographic picture in essential hypertension, have not yet made their appearance.

Finally, in evaluating the significance of the electrocardiographic changes in malignant hypertension, a tabulation was made of the age at which each patient died (Table VII). The greatest mortality was in the group 35 to 39 years old. The majority of deaths occurred after the third decade was reached, while none occurred over 50 years of age. This indicates that the electrocardiogram is a reliable guide in following the progress of malignant hypertension, and has some prognostic value. Senile degenerative changes, which may modify its usefulness, have not appeared. It is evident that the characteristic changes observed in this series are due solely to the effect of the malignant hypertension on the heart.

CONCLUSIONS

1. Low, flat, diphasic, or negative T waves and depressed S-T segments are characteristically found in Lead I, Lead II, or Leads I and II of the electrocardiogram in malignant hypertension.

2. These changes indicate the presence of myocardial damage, and are only coincidentally related to the cardiac size.

3. The cardio-thoracic ratio is not a reliable guide in interpreting the electrocardiographic picture, nor has it any prognostic value.

4. The presence of typical changes in the electrocardiogram indicates a serious prognosis for the immediate future.

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TEMPERATURE STUDIES ON INTRAVENOUS FLUIDS*

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THE purpose of this presentation is to show the temperature at which preheated or precooled fluid actually enters the vein of the patient, and not to dictate at what temperature such fluid shall be given.

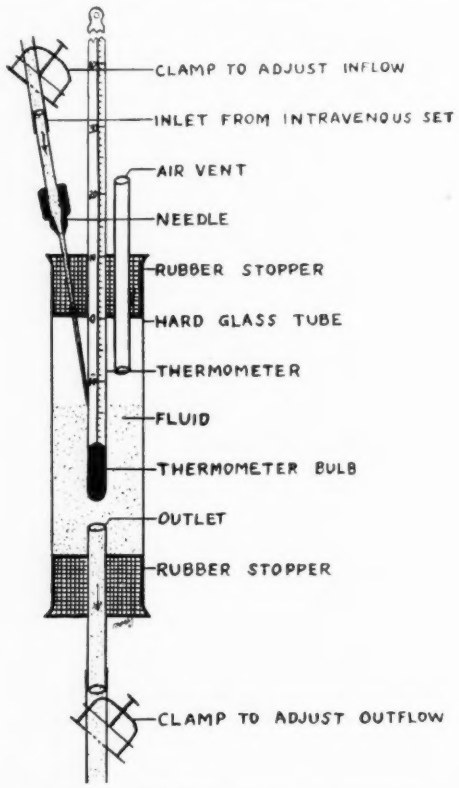

Fig. 1.—Details of thermometer assembly.

For the experimental work a standard intravenous set was used, and the fluid (normal saline) was allowed to fall one meter through regular gum rubber tubing (5 mm. diameter). A No. 20 needle was used. The needle was attached to the lower end of a Taylor Centigrade thermometer (–10 to +150° scale) by a rubber band, so that the fluid flowed directly out of the needle onto the thermometer bulb. The fluid was allowed to collect in a one liter flask, the thermometer bulb resting on the bottom of same. The flow was adjusted so that one hour was consumed in allowing one liter to run out. The temperature of the fluid was adjusted from +10 to +90° C. in 10° intervals. Three different arrangements were used originally, similar to the ones actually used in this hospital by various

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physicians. First, no extra external heat was applied after the solution was preheated. Second, two regular hot water bottles, containing 1,500 c.c. each of water at 55° C. (usual temperature of hot water in the taps in this hospital), were tied around the flask containing the fluid. Third, the tubing was "sandwiched" between two hot water bottles, 15 cm. from the needle. This last arrangement corresponds to the hot water bottles lying on the bed beside the patient's arm.

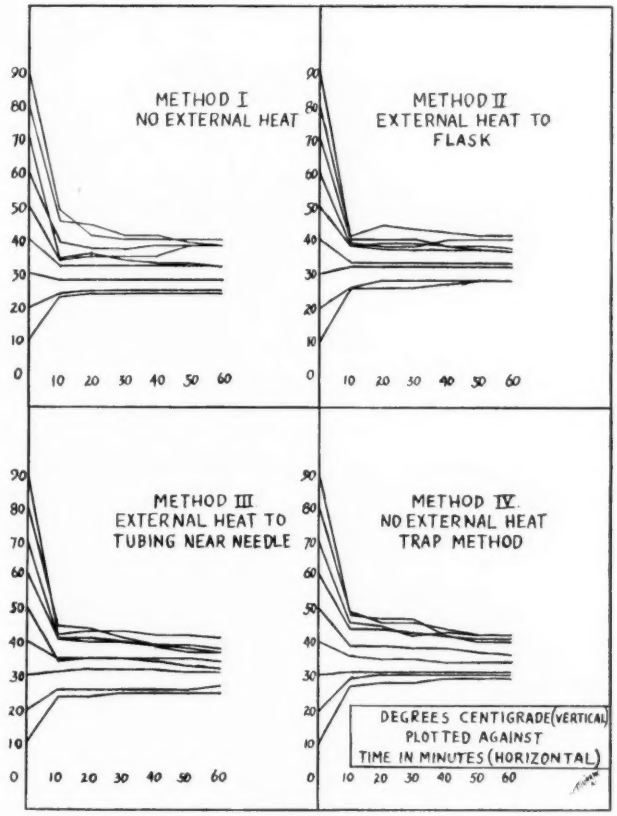

Fig. 2.

An attempt was made to read the temperature at the end of the first minute, but due to cooling by evaporation, and also due to the fact that not enough fluid had collected in the flask to cover the thermometer bulb, the readings were not considered accurate enough to include in the final report.

A fourth method was tried, in which the thermometer was inserted in a trap as shown in Fig. 1. The results are summarized in Fig. 2.

CONCLUSIONS

1. Preheated or precooled intravenous fluids tend to adjust themselves rapidly to a figure near room temperature, no matter what may be the original temperature of the original solution.

2. The customary methods of keeping intravenous fluids "warm" by the application of external heat in the form of hot water bottles are ineffectual.

A NOTE ON THE INEFFECTIVENESS OF SULFONAMIDE COMPOUNDS IN THE TREATMENT OF POLIOMYELITIS OF MICE*

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ALTHOUGH sulfanilamide and other sulfonamide compounds have been found ineffective in the treatment of experimental poliomyelitis of monkeys,[1-5] we have thought it advisable to determine whether or not they possess any therapeutic properties in poliomyelitis of mice.

Two hundred and thirty adult white mice (*Mus musculus*) were inoculated intracerebrally with 0.03 c.c. of a 5 per cent suspension in saline solution of the hind brains of mice removed after the development of flaccid paralysis following inoculation with the Lansing strain of poliomyelitis virus which has been adapted to these animals by Armstrong[6] from the eastern cotton rat (*Sigmodon hispidus hispidus*). While the virus has certain marked similarities to, as well as marked differences from, the spontaneous mouse virus first described by Theiler,[7] Lillie and Armstrong[8] have found that the pathologic changes produced by the virus in the central nervous system of mice and rats were closely similar to the reactions of poliomyelitis virus in man and monkeys.

As shown in Table I, 20, or 50 per cent, of 40 untreated controls developed paralysis and died within two to eight days after inoculation; 17, or 42.5 per cent developed paralysis and died in from ten to twenty-four days after inoculation, while only 3, or 7.5 per cent, escaped infection. All 37 of the 40 controls developing infection died within one to two days after the onset of flaccid paralysis of one or more of the legs or of the respiratory center, so that the disease in mice is so rapidly fatal after paralysis has developed that it is not well adapted for treatment purposes, although the incubation period following the intracerebral inoculation of virus is sufficiently prolonged in the majority of mice to permit the administration of repeated doses of compounds for the determination of therapeutic effects.

Sulfanilamide, sulfapyridine, and sulfathiazole, as well as two new compounds, sulfathiazoline (2-sulfanilyl 3-5 dihydrothiazole) and 2-sulfanilylaminopyrazine (No. 3032), synthesized by Raiziss and Clemence,[9] and kindly supplied by them, were administered orally in a dose of 0.005 Gm. per mouse twice daily (10:00 A.M. and 3:00 P.M.) for ten days in succession, or until paralysis and death occurred (2 to 20 doses). The first dose of each was given twenty-four hours after inoculation. As the mice averaged from 20 to 25 Gm. in weight, each dose was equivalent to about 0.2 Gm. per kilogram, or about 0.4 Gm. per kilogram per day.

*From the Research Institute of Cutaneous Medicine, Philadelphia.
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TABLE I

SULFONAMIDE COMPOUNDS IN THE TREATMENT OF POLIOMYELITIS OF MICE*

COMPOUNDS†	NO. MICE	PARALYSIS AND DEATHS; DAYS AFTER INOCULATION													NO PARALYSIS; SURVIVALS
		2	4	6	8	10	12	14	16	18	20	22	24	26	
Sulfanilamide	40	3	4	2	2	1	1	5	3	4	4	3	3	0	5 (12.5%)
Sulfapyridine	40	8	8	3	5	2	1	2	2	2	2	1	1	0	3 (7.5%)
Sulfathiazole	40	2	6	2	2	4	3	1	5	3	3	4	1	0	4 (10.0%)
Sulfathiazoline	40	4	8	2	0	6	4	0	6	6	0	2	0	0	2 (5.0%)
No. 3032‡	30	4	6	2	2	2	2	3	1	3	2	1	0	0	2 (5.0%)
Controls	40	3	8	5	4	2	2	2	2	4	1	2	2	0	3 (7.5%)

*Inoculated intracerebrally with 0.03 c.c. of a 5 per cent suspension of the Lansing strain adapted to mice (Armstrong).
†Orally in dose of 0.005 Gm. per mouse twice daily (10:00 A.M. and 3:00 P.M.), first dose twenty-four hours after inoculation.
‡2-sulfanilyl-amino-pyrazine.

As previously stated, 3, or 7.5 per cent, of 40 untreated controls survived. Of 40 mice treated with sulfanilamide, 5, or 12.5 per cent, survived; of 40 treated with sulfapyridine, 3, or 7.5 per cent survived; of 40 treated with sulfathiazole 4, or 10 per cent, survived; of 40 treated with sulfathiazoline, 2, or 5 per cent, survived, while of 30 treated with 2-sulfanilyl-aminopyrazine (No. 3032), 2, or 5 per cent, survived.

The administration of the compounds was continued after the development of paralysis, but with completely negative results, since all treated and untreated paralyzed animals died within one to two days after the onset of paralysis, permitting the administration of only one to three additional doses.

CONCLUSION

While the percentage of survivals among mice treated with sulfanilamide and sulfathiazole were slightly higher than that in the case of untreated controls, it is concluded that these compounds, as well as sulfapyridine, sulfathiazoline, and 2-sulfanilyl-amino-pyrazine, have proved ineffective in the treatment of mice inoculated intracerebrally with the Lansing strain of poliomyelitis virus (Armstrong). These results are in confirmation of the ineffectiveness of the sulfonamide compounds in the treatment of monkeys inoculated intracerebrally with the M.V. and other strains of the virus.

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CLINICAL CHEMISTRY

THE CHANGES IN THE BLOOD VOLUME PRODUCED BY DIABETIC ACIDOSIS*

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ALTHOUGH it has been recognized that diabetic acidosis is associated with hemoconcentration,[1] very few observations have been made on the volume of circulating blood under these circumstances.[2, 3] During studies on the relation of dehydration to the plasma volume, observations were made on two patients who developed diabetic acidosis under controlled conditions.

METHODS AND PROCEDURES

Blood volume determinations were made with the method of Gibson and Evans,[4] as modified by Gibson and Evelyn.[5] Venous pressure measurements were made by the direct method,[6] using 10 cm. above the level of the back as the point of zero pressure. Serum protein measurements were made by the falling-drop method described by Barbour and Hamilton.[7] The carbon dioxide combining power of the plasma was determined by the method of Van Slyke and Cullen.[8]

The patients observed were both males with diabetics who had previously been treated with diet and insulin for many years. Both were controlled on a 4,000 calorie experimental diet, consisting of 100 Gm. of protein, 265 Gm. of fat, 300 Gm. of carbohydrate, and insulin. After control values of the blood volume, venous pressure, serum protein concentration, plasma carbon dioxide combining power, and body weight were determined, insulin was discontinued. Daily urinalyses were done for sugar and ketone bodies.

RESULTS

Patient 1 was observed without insulin for nine days. Although the entire 4,000 calorie diet was regularly consumed, there was a 5 kg. weight loss during this period. There was a loss of 1.5 kg. during the first twenty-four hours coinciding with the onset of severe glycosuria. Ketonuria appeared on the fourth day and gradually increased in severity. During the night of the eighth day nausea and vomiting developed, followed in the morning by drowsiness, Kussmaul respirations, anuria, low blood pressure, clammy extremities, and

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TABLE I

PATIENT	DATE	PERIOD	WEIGHT (KG.)	VENOUS PRESSURE (MM. WATER)	TOTAL BLOOD VOLUME	PLASMA VOLUME	TOTAL BLOOD VOLUME/ KG. BODY WT.	PLASMA VOLUME/ KG. BODY WT.	HEMATOCRIT	SERUM PROTEIN GM./100 C.C.	CARBON DIOXIDE COMBINING POWER	KETONURIA
1	2/18/41	Control	62.3	100	3,745	2,370	60.1	38.1	36.75	6.36	67.3	Negative
	2/18/41	Without insulin										Negative
	2/19/41	Without insulin	60.8									Negative
	2/20/41	Without insulin	59.5								64.2	Negative
	2/21/41	Without insulin	60.5								62.6	Positive
	2/25/41	Without insulin	58.6									Positive
	2/26/41	Without insulin	57.3		2,885	1,500	52.0	26.2	48.00	7.72	14.0	Positive
	4/23/41	Control	61.4	110	3,560	2,135	58.0	34.8	40.00	6.18	62.5	Negative
2	5/ 6/41	Control	72.2	130	6,100	3,660	84.4	50.7	40.00	6.71	57.0	Negative
	5/ 6/41	Without insulin										
	5/10/41	Without insulin	71.8	136	6,020	3,600	83.8	50.1	40.20	6.89	60.0	Positive
	5/15/41	Without insulin	69.4	134	5,820	3,240	83.8	46.7	44.30	7.47	47.5	Positive
	5/20/41	Without insulin	67.1	100	5,200	2,860	78.4	42.6	45.00	8.05	26.0	Positive
	6/10/41	Control	73.2	140	6,360	3,560	86.9	48.6	44.80		52.2	Negative

marked collapse of the veins. The plasma carbon dioxide combining power at this time had fallen to 14.0 volumes per 100 c.c. The arterial blood pressure, usually 130/80, was now 90/60. The veins were collapsed.

There was a severe reduction of 860 c.c. (23 per cent) in the total blood volume due entirely to a 36 per cent decrease in plasma volume. In spite of the loss of 5 kg. of weight, there was a marked decrease in the blood volume and plasma volume per kilogram of body weight from 60 c.c. to 52 c.c. (13.4 per cent) and from 38 c.c. to 26 c.c. (31.2 per cent), respectively. The hematocrit and serum protein concentrations were increased 30.6 per cent and 21.4 per cent, respectively. These changes are recorded in detail in Tables I and II. There was a prompt recovery within eight hours following the intravenous administration of 4,000 c.c. of 5 per cent glucose in normal saline, insulin, sodium bicarbonate, and forced fluids by mouth.

Patient 2, who was not as severe a diabetic as Patient 1, was observed without insulin for fifteen days. Although the high caloric intake was maintained, there was a 5.1 kg. loss in weight, most of which occurred after the plasma carbon dioxide combining power had decreased.

TABLE II

PERCENTILE CHANGES

PATIENT	% CHANGE BODY WEIGHT	% CHANGE IN TOTAL BLOOD VOLUME	% CHANGE IN PLASMA VOLUME	% CHANGE IN TOTAL BLOOD VOLUME/KG. BODY WT.	% CHANGE IN PLASMA VOLUME/KG. OF BODY WT.	% CHANGE IN HEMATOCRIT	% CHANGE IN CONCENTRATION OF SERUM PROTEINS
1	-8.0	-23.0	-36.2	-13.4	-31.2	+30.6	+21.4
2	-5.7	-14.7	-21.8	- 7.1	-15.9	+12.5	+20.0

By the fifteenth day there was an 800 c.c. (21 per cent) decrease in plasma volume and a marked reduction in total blood volume and plasma volume per kilogram body weight from 84 c.c. to 78 c.c. (7.1 per cent) and from 51 c.c. to 43 c.c. (15.9 per cent), respectively. The loss of blood volume was largely at the expense of the plasma volume; the hematocrit and serum protein concentrations were increased 12.5 per cent and 20 per cent, respectively. The venous pressure was reduced from 130 mm. to 100 mm. of water (23 per cent). These changes are recorded in detail in Tables I and II. The patient promptly recovered with the administration of insulin, fluids, and glucose by mouth.

DISCUSSION

The data presented here indicate that the blood volume is significantly decreased in diabetic acidosis, the reduction being entirely at the expense of the plasma volume. Both the total blood volume and the plasma volume per kilogram body weight were also reduced. There was an increase in the hematocrit and plasma protein concentration that closely paralleled the decrease in plasma volume. These results are in agreement with those of Chang, Harrop, and Schaub,[2] who used the carbon monoxide method to determine blood volume and who found a reduction in the blood volume per kilogram body weight and an average decrease in plasma volume of 19 per cent. Peters[1] reported details

on 13 patients who had an average decrease of 16 per cent in the concentrations of serum proteins with recovery from diabetic acidosis. The large decreases in plasma volume in man, as determined by the dye method and the carbon monoxide method, as well as the changes in hematocrit and serum protein, indicate that the plasma volume shares an important part in the dehydration associated with diabetic acidosis.

Sundermann and Dohan,[3] using vital red dye for the measurement of blood volume, in a study of ketosis in 6 pancreatectomized dogs, found an average decrease of 10 per cent in total serum volume and an actual increase of 29 per cent in the serum volume per kilogram body weight. None of these dogs, however, developed a marked acidosis, as measured by the carbon dioxide content of plasma. It is possible that under these circumstances the acidosis was not sufficiently severe or prolonged to cause a greater depletion of the extracellular fluid. Atchley, Loeb, and others,[9] in a study of the electrolyte changes in two patients with diabetes, found that with the withdrawal of insulin there was an increase in the excretion of both potassium and sodium which would indicate a loss of both intracellular and extracellular fluid. They have pointed out that the initial electrolyte and water loss was dependent upon the sudden appearance of marked glycosuria.

The demonstration of the marked fall in blood volume in man associated with the decrease in venous pressure may well explain the cause for the peripheral circulatory collapse often seen in severe diabetic acidosis. It has been observed[10] that the diminution in plasma volume produced by mercurial diuretics or ammonium chloride acidosis is associated with a nearly proportionate decrease in venous pressure in normal persons. It has been established that changes in venous pressure in dogs and man will produce significant alterations in the cardiac output.[11-15] Thus in diabetic acidosis the low blood volume and its accompanying fall in venous pressure may produce a decrease in the cardiac output. The low arterial pressure, anuria, clammy extremities, and the clinical picture of peripheral circulatory collapse seen in diabetic acidosis may, therefore, be the result of the decrease in the volume of circulating blood.

The marked reduction in blood volume in diabetic acidosis, particularly when associated with peripheral circulatory collapse, should indicate immediate replacement of the blood volume as an important step in the management of this type of patient. Although there may be an increase in the concentration of serum proteins at the time of the acidosis, the parenteral administration of normal saline or glucose may not necessarily be expected to increase the plasma volume immediately,[1] since the fluid may be lost to dehydrated tissues. Blood transfusion, or better still serum transfusion, should be especially helpful in the replacement of plasma volume and the relief of the circulatory collapse.

CONCLUSIONS

1. Diabetic acidosis observed in two patients was associated with a marked reduction of blood volume due to the loss of plasma volume and a fall in blood volume per kilogram body weight.

2. Associated with the fall in blood volume there was also a significant fall in venous pressure.

3. It is emphasized that the circulatory collapse sometimes found in diabetic acidosis is in part at least the result of the accompanying decrease in blood volume and venous pressure.

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THE VALUE OF BLOOD CHLORIDE AND SODIUM DETERMINATIONS IN THE DIAGNOSIS OF DEHYDRATION*

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IN A typical case the syndrome of dehydration is not difficult to recognize. The patient shows dryness of the mouth and tongue; shocklike weakness and apathy; diminished skin turgor; sunken, softened eyeballs; lowered blood pressure, associated frequently with a rapid pulse rate and other signs of circulatory distress, and marked diminution in urinary output, which occasionally approaches anuria. Frequently, however, the clinical picture is not so straightforward as thus presented. It may be obscured by other symptoms associated with the disease producing the dehydration, or it may even be produced by the therapeutic measures undertaken to control the disease. Not infrequently, because the scanty urine may contain some albumin and a number of casts and even red blood cells, and may be associated with nitrogenous retention, the condition is diagnosed as acute nephritis. This diagnosis usually causes a paralysis of all efforts at controlling the dehydration.

Since severe clinical dehydration is always associated with a loss of salt, and since this loss is reflected in the concentration of chloride and sodium in the blood,[1] it should be possible to recognize the condition by the appropriate blood chemical determinations. It is the purpose of this paper to present evidence that determinations of whole blood chloride are usually misleading in the information they impart concerning the state of hydration, because the whole blood chloride concentration varies with the degree of the anemia. It will be shown also that, though serum chloride values are not affected by the red blood cell concentration and though they are a more satisfactory gauge of dehydration than whole blood chloride values, they are also at times unreliable; for the serum chloride may be normal or even higher than normal in many cases of excessive water loss. On the other hand, evidence will be offered to show that a lowering of serum sodium concentration always accompanies severe dehydration and that its determination, now feasible in all clinical laboratories, should be used in conjunction with serum chloride analysis in the diagnosis of dehydration.

METHODS

To determine the influence of red blood cell concentration upon the whole blood and serum chloride levels, a study of the distribution of chloride between

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cells and serum (or plasma) was made in 80 persons. These persons were either healthy medical students or technicians or were ambulatory dispensary patients with varying degrees of anemia. No cases of pernicious anemia or other types of hyperchromatic anemia were chosen. No patient had any clinical or laboratory evidence of disturbance of water or electrolyte balance.

For the analysis, blood was drawn from an arm vein with a minimum of stasis into dry syringes. The portion for whole blood was immediately transferred to a tube containing potassium oxalate. For the determination of red blood cell volume percentage, Van Allen[2] hematocrit tubes were filled to the mark and diluted with 1.3 per cent sodium oxalate. The tubes were sealed with wide rubber bands and then centrifuged at 2,500 r.p.m. for at least twenty minutes, until the column of red blood cells no longer diminished in height. Blood for serum analysis was transferred under oil to centrifuge tubes, stoppered and allowed to clot, and then centrifuged for ten minutes. The serum was separated and recentrifuged to remove the last trace of red blood cells. Both whole blood and serum chloride determinations were made by the microphotoelectric iodate method of Sendroy,[3] as modified by one of us (W. S. H.).[4] All analyses were made in duplicate and averaged. Hematocrit estimations were made in triplicate. The red blood cell concentration was estimated from the equation

$$R = \frac{100\ W - (100 - H)\ S}{H} \qquad (1)$$

where R and S are the red blood cell and serum chloride concentrations, respectively; W, the whole blood concentration; and H, the volume percentage of red blood cells or the hematocrit reading.

For the determinations in the later clinical studies in this paper mentioned in Table II, the following methods were employed: sodium, Hoffman and Osgood;[5] carbon dioxide-combining power, Van Slyke;[6] protein and nonprotein nitrogen, Hoffman and Osgood;[7] hemoglobin, Sheard and Sanford.[8] In all the photoelectric methods the Cenco-Sheard-Sanford Photelometer was used. The average normal concentration of serum sodium was found to be 143 meq. per liter, the range being 138 to 148 meq. per liter.

WHOLE BLOOD CHLORIDE

For the sake of brevity, the results of the analyses are expressed in frequency charts in Fig. 1 and as scatter curves in Fig. 2. Serum chloride concentration (Fig. 1*a*) varied from 100 to 110 meq. per liter, the great majority of values falling between 102 and 107 meq. per liter. The average serum chloride concentration was 104.4 meq. per liter, with a standard deviation of ±2.0 meq. per liter (610 ± 12 mg. NaCl per 100 c.c.). The solid circles in Fig. 1 represent cases of anemia, that is, patients with hematocrit readings less than 40 per cent. It can be seen that there is no difference between the distribution of the serum chloride values of anemic persons and those with normal red blood cell volume. All the values fit into a compact probability curve. Similarly, the red blood cell chloride values are independent of the hematocrit reading (Fig. 1*b*). Their range is relatively wider than that of the serum

chloride values, and the probability curve arrangement is not so definite. However, this fault can be explained by the greater error of the calculation of the red blood cell chloride. An error of 1 meq. in either whole blood or serum chloride produces an error of 2 to 5 meq. in the red blood cell chloride concentration, depending upon whether the hematocrit reading is high or low. Nevertheless, the distribution of the values for anemia cases is the same as that for normal cases. The range is from 47 to 57 meq. per liter, with a mean value of 52.3, and a standard deviation of ±2.5.

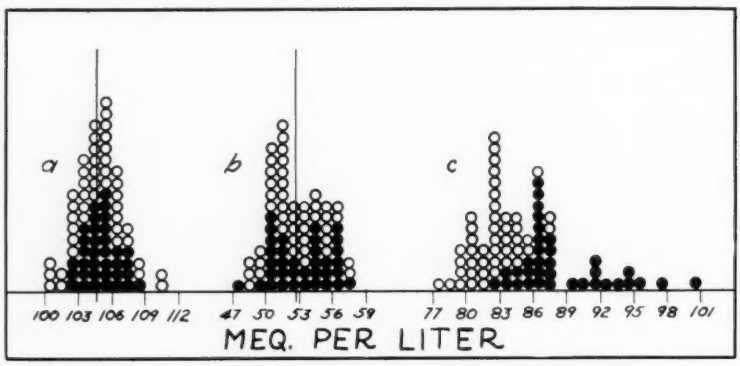

Fig. 1.—Frequency distribution of chloride concentrations in *a*, serum *b*, red blood cells and *c*, whole blood in 80 cases. Solid circles represent cases of anemia, that is, hematocrit readings of less than 40 per cent.

On the other hand, the values for whole blood chloride (Fig. 1*c*) are much more widely distributed than those for serum or red blood cells. The range is from 77 to 100 meq. per liter. The average value is 84.6 meq. per liter, and the standard deviation is ±4.6 meq. per liter. However, the values fit so poorly into any probability curve that the mean value and the standard deviation have very little significance. The anemia cases are distributed all to the right side of the chart, indicating that the whole blood chloride is dependent upon the degree of anemia. If there had been more severe anemia cases, there would undoubtedly have been more weighting to the right, and an increase in the standard deviation.

If the red blood cell and serum chloride concentrations are independent of the hematocrit reading, then the whole blood chloride *must* vary with the hematocrit reading. This can be seen from equation (1), which may be rewritten as

$$W = S - \frac{S - R}{100} H \qquad (2)$$

where S and R (the serum and red blood cell chloride concentrations, respectively) are regarded as constants, and W and H (the whole blood chloride and the hematocrit) are variables. The lower the value of H, the higher the value of W. When the hematocrit becomes zero, the whole blood chloride equals the serum chloride, a conclusion which is obvious without reference to the equation. Choosing S as 104.4 meq. per liter, and R as 52.3 meq. per liter, equation (2) becomes the line AB in Fig. 2*a*, where W is the ordinate and H is the abscissa. This is the curve of expected values for whole blood chloride,

based on the assumption of the constancy of the serum and red blood cell chloride concentrations. The curve is an oblique line passing through the base (H = O) at W = 104.4. The observed values for whole blood chloride, plotted against the hematocrit with the same coordinates as the curve AB, form the scatter curve which follows closely the theoretical curve. The deviations from the points on the theoretical curve are no wider than those in the scatter for serum chloride plotted along with the theoretical equation, S = 104.4 (line AC, Fig. 2*b*).

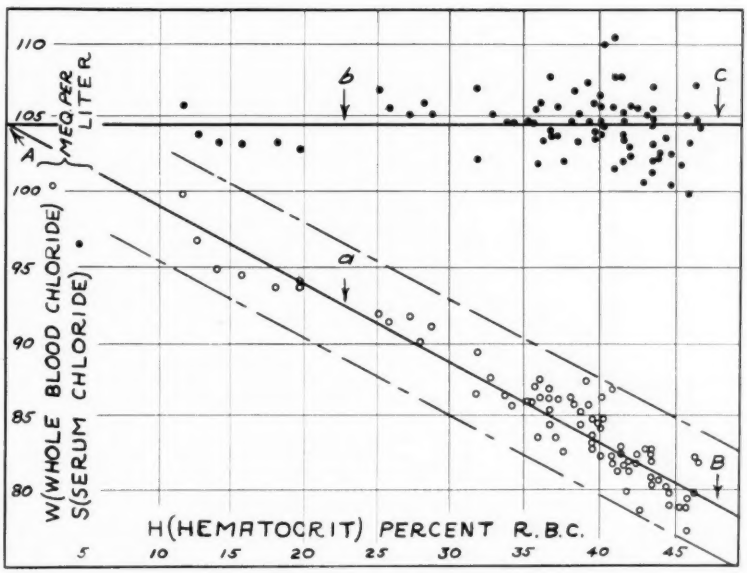

Fig. 2.—*a*, Scatter curve showing the relationship between whole blood chloride concentrations and the hematocrit readings in 80 cases. The line AB represents the theoretically expected curve. *b*, Scatter curve showing the relationship between serum chloride concentrations and the hematocrit readings in the same 80 cases. The line AC represents the theoretically expected curve.

Thus these data offer statistical confirmation of the proposition first stated by Norgaard and Gram[9] that the serum and cell chloride concentrations are independent of the degree of anemia, but that the whole blood chloride concentrations must necessarily vary with the red blood cell volume percentage. Failure to recognize this fact has made whole blood chloride determinations worthless in most clinical laboratories. It would probably be better that the determination be abandoned altogether for clinical purposes. But where it is carried out, the expected normal range must be determined from a chart such as Fig. 2*a*. For this purpose, Table I has been constructed from the data in Fig. 2*a* and from the hemoglobin concentrations determined in the 80 cases. The range of probable values for any given hematocrit was determined by drawing two lines parallel to the theoretical curve AB, so as to include all the observed values. Table I should be of aid in recognizing low blood chlorides in those cases where such a finding confirms the diagnosis of dehydration. The following two cases illustrate the failure to interpret properly whole blood chloride determinations.

TABLE I

VARIATIONS OF WHOLE BLOOD CHLORIDE WITH THE HEMOGLOBIN CONCENTRATION AND RED BLOOD CELL VOLUME IN BLOOD OF PATIENTS WITH NO ELECTROLYTE DISTURBANCE

HEMOGLOBIN GM./100 C.C.	HEMATOCRIT % R.B.C.	WHOLE BLOOD CHLORIDE			
		RANGE OF NORMAL		EXPECTED MEAN	
		MG. NACL/100 C.C.	MEQ./LITER	MG. NACL/100 C.C.	MEQ./LITER
13-16	40-47	456-509	78-87	479	82
11-13	35-40	474-526	81-90	497	85
9-11	30-35	491-544	84-93	514	88
8-9	25-30	503-555	86-95	532	91
7-8	20-25	520-573	89-98	544	93
3-7	10-20	532-602	91-103	567	97
0 (Serum)	0	585-643	100-110	608	104

CASE 1.—A girl of 21 was brought to the hospital suffering from fever, jaundice, repeated vomiting, and oliguria, following an attempted abortion. The patient did abort, but her condition grew worse. The urine was scanty and showed some albumin, red blood cells, and several casts, and there was some nitrogenous retention. A diagnosis of acute nephritis was considered. The hemoglobin was 10 Gm. per 100 c.c., and the whole blood chloride was 450 mg. of NaCl per 100 c.c. This value was regarded as within normal limits, since the range of whole blood chloride is usually given as 450 to 500 mg. of NaCl per 100 c.c. When it was pointed out that the expected whole blood chloride for such a degree of anemia was 514 mg. of NaCl per 100 c.c. and that the probable lower limit for normal was 491 mg. of NaCl per 100 c.c. a serum chloride analysis was made. The serum chloride concentration was 538 mg. of NaCl per 100 c.c. or 92 meq. per liter, a value 11 per cent below the average normal. The patient was given 4 liters of physiologic saline intravenously within twenty-four hours. She began to urinate and made a complete recovery.

CASE 2.—A man aged 65 years, five days after a prostatectomy, developed secondary hemorrhage. Following this accident, the quantity of urine diminished rapidly, and the blood chemical analysis showed increasing nitrogenous retention. The patient had been on a low salt diet previous to the operation and had little salt following the operation. The condition was diagnosed as uremia following renal failure. The hemoglobin concentration was 8.9 Gm. per 100 c.c., and the whole blood chloride concentration was 465 mg. of NaCl per 100 c.c., which was thought to be well within normal limits. But with this degree of anemia, the whole blood chloride should have been within the limits of 503 to 555 mg. of NaCl per 100 c.c. When this fact was made clear, intravenous saline therapy was instituted, and improvement soon followed. With adequate urinary output the blood nonprotein nitrogen concentration soon decreased to normal, and the patient eventually recovered.

SERUM CHLORIDE VERSUS SERUM SODIUM

The foregoing data unequivocally indicate the diagnostic advantages of serum chloride determinations over those of whole blood chloride. In many uncomplicated cases of dehydration, the serum chloride is sufficiently lower than normal to confirm the clinical diagnosis. However, this is not always true. The data assembled in Table II from our own cases indicate that serum chloride may be low, normal, or even high in proved cases of dehydration. The 15 cases in Table II were chosen to illustrate the findings in dehydration associated with a variety of disease processes. In all these cases the clinical symptoms of dehydration were pronounced, and clinical improvement followed immediately upon the intravenous injection of sufficient physiologic saline solution (usually along with Hartmann's solution) to restore the electrolyte balance to normal. The quantity of fluid required for an adult usually was about 4,000 c.c. in the first twenty-four hours.

TABLE II

BLOOD CHEMICAL FINDINGS IN 15 CASES OF DEHYDRATION

CASE	DISEASE	HEMOGLOBIN GM./100 C.C.	HEMATOCRIT % R.B.C.	SERUM CHLORIDE MEQ./LITER	WHOLE BLOOD CHLORIDE MEQ./LITER	SERUM SODIUM MEQ./LITER	CO_2-COMBINING POWER C.C./100 C.C.	SERUM PROTEIN GM./100 C.C.	N.P.N. MG./100 C.C.	REMARKS
3	Chronic glomerulonephritis									
	a. Before treatment	10.0	27.5	97.4	82.2	130	47	5.90	89	Marked vomiting
	b. After NaCl therapy	8.5	24.2	106.6	92.0	141.5	55	5.50	78	
4	Acute glomerulonephritis	12.9	38	90.2	65.2	132.5	57	5.66	140	Marked oliguria and azotemia
5	Chronic glomerulonephritis	9.2	27.0	123.2	106	135	27	4.06		Marked acidosis due to prolonged NH_4Cl ingestion
6	Acute glomerulonephritis	7.4		87.6		116	46	5.28	108	Anuria following eclampsia and delivery
7	Chronic glomerulonephritis	6.6	21.0	107	94.7	134	34	6.75	148	Polyuria, vomiting
8	Malignant nephrosclerosis	4.8	14.5	103.5	95.0	134	23	5.31	240	Hemorrhage, vomiting, uremia
9	Acute hepatitis			96.5		125	45	9.34		Fever, vomiting, diarrhea
10	Acute gastroenteritis			111		127	38	9.12		Dysentery
11	Diabetic acidosis			101		128	23	7.48		Polyuria
12	Pyloric obstruction			91		136	84	8.10		Vomiting, oliguria
13	Gastrocolic fistula	12.0	40.0	85		133	88	6.78	40	Diarrhea
14	Addison's disease	12.5	38.5	100		128	52	6.60	62	Polyuria, vomiting
15	Addison's disease	11.8	37.8	110		134	48	6.38	56	Polyuria, vomiting
16	Diabetic acidosis	13.5	41.0	96	82.0	128	28	6.50	44	Polyuria, vomiting
17	Mercurial nephrosis	12.8	38	85	68.2	117	44	8.40	86	Vomiting, diarrhea, oliguria

In 8 of the 15 cases the serum chloride values, as well as the serum sodium values, were lower than normal, but in the remaining cases the chloride values were normal, at the upper limit of normal, or even higher than normal. When vomiting is a prominent symptom in the disease producing dehydration, chloride is usually depleted at the same rate as sodium. In those cases where partial restitution of electrolyte balance is attempted either unwittingly or therapeutically by ingestion of sodium chloride, chloride may rise to normal or above normal while the sodium concentration still remains low. This is particularly likely to occur in Bright's disease and in Addison's disease, when the kidneys, because of either organic or functional derangement, cannot readily excrete the excess chloride. Case 5 illustrates the development of dehydration and acidosis during an uncontrolled intake of ammonium chloride. Here, dehydration was present in spite of extremely high serum chloride concentration, yet the serum sodium value properly reflected the clinical state.

In all 15 cases the serum sodium concentration was lower than the minimal normal value of 138 meq. per liter. The deviation from normal was not very great, if one uses as a criterion the changes that can occur in urea or glucose concentrations. But even a 5 per cent drop below the average normal value indicates marked dehydration, for in the early stages of dehydration the sodium concentration still remains within normal limits. Even in the alkalosis of pyloric obstruction and gastrocolic fistula (Cases 12 and 13) there was a sodium deficit. It was not as great as the chloride deficit, but it determined the degree of dehydration in these cases. In the first hours of pyloric vomiting the chloride may drop rapidly below normal, but not until the sodium loss, in the vomitus and in the urine, produces a serum sodium concentration lower than normal, is severe dehydration present.

When, as in Case 6, the serum sodium is as low as 116 meq. per liter, it is scarcely compatible with life. Along with the usual symptoms of dehydration, there is marked oliguria and azotemia. The symptoms and signs may be difficult to distinguish from true uremia. The patient, in this case, had had labor induced because of eclampsia. At the time of the blood chemical analysis she had scarcely urinated at all for several days. She was bloated, had pallid facies, was weak and apathetic, and was obviously in serious condition. The blood nonprotein nitrogen concentration was 108 mg. per 100 c.c., and the blood pressure was 170 mm. systolic and 100 mm. diastolic. In spite of the risk of recurrence of convulsions saline injections were recommended. The patient was given 3,000 c.c. of physiologic saline solution intravenously during the first twenty-four hours, and 2,000 c.c. the following day. The blood pressure rose slightly, but no convulsions occurred. The urinary output was 1,200 c.c. on the day following onset of treatment and rose to 3,000 c.c. daily. The nonprotein nitrogen dropped to normal, and the patient recovered completely in several weeks.

The data in Case 3 illustrate the changes in the blood chemistry after adequate water and salt therapy. The serum sodium, chloride, and carbon dioxide-combining power rose to normal value. At the same time the increase in plasma volume produced a dilution of hemoglobin, red blood cells, serum protein, and nonprotein nitrogen. Thus, though it was true that the period

of dehydration was associated with hemoconcentration, this could not have been ascertained at the time by analysis for hemoglobin, red blood cell volume, or serum protein, for all these values were well below normal. Yet the serum sodium and chloride indicated the true condition. (It should be noted that the whole blood chloride was within so-called normal limits.) Only in 4 of the 15 cases was the serum protein above the normal limits, even though hemoconcentration was undoubtedly present in all these cases.

The determination of serum sodium, it has been seen, may be of lifesaving importance. It should not necessarily replace the determination of serum chloride. The two analyses, along with that of the carbon dioxide-combining power, should be performed in all cases of suspected water and acid-base disturbance. The three determinations together give adequate information in severe cases of dehydration, not only in regard to the volume and mineral content of the extracellular fluids, but also with respect to the degree and type of acid-base balance disturbance. If, for example, the sodium and chloride are equally lowered and yet the carbon dioxide-combining power is low, then the acidosis is due to the retention of acid ions other than chloride. In nephritis these ions are almost always phosphate and sulfate, but in other diseases they are usually organic acids—acetoacetic and hydroxybutyric acids in diabetes and starvation; lactic acid in cardiac failure and in convulsions.

Moderate or incipient dehydration is not associated with changes in serum chloride or sodium. There is at present no laboratory aid for the detection of such early dehydration. However, if the dehydration is not severe enough to have produced electrolyte changes, it is seldom a factor in the differential diagnosis. Morever, it is usually not difficult to recognize the condition by clinical observation. As Simeone[10] has recently pointed out: "Long before ionic changes are demonstrable in the blood, the withdrawal of interstitial fluid causes a diminution in the blood flow through the salivary glands, the secretions from which are thereupon impoverished. Thus the best index of early dehydration is the old clinical observation of dryness of the buccal and oral mucous membranes. Dysphagia is usually present. . . . The value of blood chemical determinations is not to be belittled, however; they at least demonstrate the degree of dehydration, and the nature of the shift in electrolyte pattern serves as a guide for replacement therapy."

SUMMARY

A study of the distribution of chloride between red blood cells and serum was made in 80 persons who had varying red blood cell volume percentages but who had no evidence of dehydration or electrolyte imbalance. The serum chloride ranged between 100 and 110 meq. per liter, with a mean value of 104.4 and a standard deviation of ±2.5. The red blood cell chloride concentration was also independent of the anemia. The whole blood chloride, however, varied directly with the red blood cell volume in a predictable manner. From the scatter curve of variations of the whole blood chloride with the hematocrit reading, a table was prepared which indicated the expected whole blood chloride value for any particular hemoglobin concentration or hematocrit reading. It was shown that failure to interpret whole blood chloride concentrations

according to such a table has prevented clinicians from recognizing dehydration, particularly when such dehydration was the cause of oliguria and nitrogenous retention.

In a study of 15 cases of severe dehydration in a variety of disease processes, serum sodium was found always lower than the minimal normal of 138 meq. per liter. In these cases, serum chloride, though at times correspondingly low, was occasionally within normal limits or even higher than normal.

The conclusion was reached that a proper evaluation of the water and mineral content of the body in dehydration, both from the point of view of diagnosis and therapy, can be achieved by analysis of the serum for sodium, chloride, and carbon dioxide-combining power.

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THE EXPERIMENTAL PRODUCTION OF HYPOALBUMINEMIA BY FASTING*

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ALTHOUGH the causal relationship of a protein-deficient diet and nutritional edema has been known since World War I, hypoproteinemia was not recognized as a crucial phenomenon, largely because there were few determinations of plasma protein in dietary deficiency until the extensive clinical observations of Peters and his co-workers,[1] beginning about 1926. A summary of most of their findings and a review of the literature was published by them in 1933.[2] Prominent among the contributions of Peters was, first, the fact that the fall in serum protein in malnourished patients was confined to the albumin fraction, the globulin remaining relatively unaffected; second, that the fall in serum protein might be masked by dehydration, the lowered value not becoming evident until the dehydration was corrected; third, various infections often produced a rise in serum globulin, thus creating a normal total serum protein, even though there was a depletion in the albumin fraction.

Experimental hypoproteinemia was induced with a protein-deficient diet by Frisch, Mendel, and Peters,[3] who were able to lower the serum protein in white rats to 3.0 Gm. per 100 c.c. Much more extensive observations on the dog were made by Weech and his co-workers,[4] who used a low protein diet, consisting of carrots, sucrose, lard, rice, cod-liver oil, and a salt mixture. Confirming the clinical observations of Peters, they noted that the fall in serum protein was confined to the serum albumin, the globulin fraction remaining unchanged. A second, and very important observation, was the fact that the fall in serum albumin began at once and continued progressively during the first month or more the animal was on the diet.

Though the influence of a complete fast on the concentration of serum protein has received considerable attention, the findings are much less conclusive. As early as 1882 Burckhardt[5] found that the concentration of total protein fell slightly when dogs were fasted for four days. Hanson,[6] in 1918, reviewed the earlier literature and concluded that "a wide disparity in the results obtained by different workers is the rule rather than the exception." Hanson himself studied six rabbits during alternate periods of fasting and feeding, the longest period of fasting being six days. Though no very marked differences were noted, there was a tendency for the albumin concentration to be slightly lower after the longer periods of fasting. Bloomfield[7] and Torbert[8] studied rats which were fasted, killed at intervals, and the serum protein determined. Though the total protein concentration dropped initially,

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the findings in general were not very conclusive. However, Torbert noted that the initial drop was in the albumin fraction; he found, furthermore, that the blood volume decreased during the experiment so that the total circulating protein diminished, even though the concentration remained relatively unchanged. The same finding was reported in a carefully controlled experiment by Holman, Mahoney, and Whipple[9] in a dog that was fasted for twenty-four days. These workers concluded that "fasting usually does not modify the total protein concentration in the plasma, and the albumin-globulin ratio may not be changed, but fasting does cause conspicuous shrinkage of the blood plasma volume and, therefore, the total amount of circulating protein may decrease to 70, 60, or even 50 per cent of normal."

During a complete fast there is, of course, an increased breakdown of protein in order to supply calories, thus leading to a release of large amounts of nitrogen, much of which may remain as amino acids. The idea has been expressed that this extra nitrogen might spare serum protein depletion or even lead to its regeneration. Indeed Weech and his collaborators,[10] and Melnick and his collaborators,[11] each found that during a period of starvation, there was an increase in the concentration of serum protein in a dog with chronic hypoproteinemia.

EXPERIMENTAL PROCEDURES

Two series of experiments were carried out in 15 dogs. In Experiment I 5 dogs were given by gavage for three weeks a solution containing Karo syrup, Ringer's solution, and vitamin B complex,* and were allowed water ad libitum; the energy value was 500 calories per day, due entirely to carbohydrate, and nitrogen was 0.07 Gm. per day. In Experiment II 10 dogs were fasted completely; water was allowed ad libitum, but 3 did receive in addition Ringer's solution by gavage. Samples of heparinized blood were removed at the beginning of the experiment and each week thereafter by puncturing the jugular vein and removing a sample with as little stasis as possible. The red cell volume was determined after centrifugalization, and the supernatant plasma used for the determination of albumin and globulin, separation being carried out by the method of Campbell and Hanna.[12] Determinations of the nitrogen were carried out by a micro-Kjeldahl, as described by Sobel, Yuska, and Cohen.[13]

EXPERIMENTAL FINDINGS AND COMMENT

The present findings show that hypoalbuminemia occurs on a protein- and fat-deficient diet regardless of whether or not there is any caloric intake. Thus the serum albumin concentration declined both in the 5 dogs given a solution containing carbohydrate, Ringer's solution, and vitamin B (Table I), as well as in the 10 animals fasted completely (Table II). Moreover the changes were progressive and started from the very beginning of the experiment.

The average fall in the albumin was twice as great in the dogs receiving calories as in those completely fasted (29 per cent as compared with 14 per cent). Inferences cannot be drawn from this striking difference, which was due in part at least to hemoconcentration occurring in the fasted dogs, as

*Labco was obtained from The Borden Company, Bainbridge, N. Y.

TABLE I

HYPOALBUMINEMIA DURING A NONPROTEIN DIET

(Intake: Karo syrup, Water, Electrolyte, and Vitamin B)

A = Plasma albumin, grams per 100 c.c. H = Hematocrit as per cent.

G = Plasma globulin, grams per 100 c.c.

DOG	INITIAL			ONE WEEK			TWO WEEKS			THREE WEEKS		
	A	H	G	A	H	G	A	H	G	A	H	G
Z-10	3.90	50	3.00	2.84	44.6	2.75				2.08	39.7	3.52
Z-24	3.65	44	2.54	2.96	46.0	2.83				2.76	44.3	3.62
Z-14	2.58	56	3.28	2.05	55.8	3.22				1.99	52.4	3.31
T-67	3.03	57	2.80	2.41	53.7	3.36				2.22	52.0	3.10
T-56	3.79	52	2.90	3.20	47.9	3.17				3.10	50.0	2.76
Averages												
Albumin	3.39			2.62						2.43		
Hematocrit		51.8			49.6						47.7	
Globulin			2.90			3.07						3.26

TABLE II

HYPOALBUMINEMIA DURING COMPLETE STARVATION

(Intake confined to water *ad lib.* except Dogs 62, E6, and E2 who also received some Ringer's solution)

A = Plasma albumin in grams per 100 c.c. H = Hematocrit as per cent.

G = Plasma globulin, grams per 100 c.c.

DOG	INITIAL			ONE WEEK			TWO WEEKS			THREE WEEKS		
	A	H	G	A	H	G	A	H	G	A	H	G
F-8	3.39	45.1	2.90	3.03	46.6	3.08	2.90	44.3	2.77	2.94	42.4	2.58
56F2	*2.94*	*48.2*	*2.25*	*3.33*	*53.1*	*2.61*	3.13	54.1	2.39	2.56	57.2	2.38
F-23	2.61	42.9	4.87	2.53	42.1	5.58	2.28	44.4	5.34	2.34	48.8	5.37
Z-10	*2.88*	*47.1*	*3.12*	*3.28*	*51.4*	*3.30*	2.93	57.9	3.12	2.66	51.7	3.05
52	3.14	53.5	3.52	2.89	48.2	3.37	2.68	49.5	3.20	2.44	46.2	4.12
Z-8	3.92	51.4	3.02	3.64	54.2	2.73	3.28	56.0	2.57	3.31	57.0	2.73
59	3.26	51.1	2.17	3.22	51.0	2.66	2.84	49.2	2.52	2.76	43.2	2.52
62	3.73	52.6	3.32	3.03	49.6	2.92	*2.96*	*49.7*	*2.71*	*3.48*	*61.0*	*3.63*
E6	2.65	48.8	2.75	2.46	48.3	2.99	2.28	47.9	3.04	2.23	44.0	3.01
E2	3.64	46.7	2.78	3.36	44.8	2.70	3.10	49.8	2.65	3.07	50.4	2.56
Averages												
Albumin	3.22			3.07			2.83			2.77		
Hematocrit		48.7			48.9			50.2			50.1	
Globulin			3.07			3.19			3.03			3.19

indicated by comparing the behavior of the hematocrit values in the two groups. Thus, while the average red blood cell volume fell in Experiment I, there was a slight increase in Experiment II. Though there was considerable individual variations in the group of fasted animals, as can be seen by studying each of the experiments in Table II, the increase in the red blood cell volume was so pronounced in several instances that the fall in serum albumin not only was masked but even gave way to a rise. (See the italicized values in Table II.) This effect of dehydration on the concentration of serum albumin was first observed in human beings by Peters and co-workers,[1] as already mentioned.

One can only speculate on the influence exerted by caloric intake on the magnitude of the albumin loss in view of the striking influence of a complete fast on the red blood cell volume as just pointed out. Whether the protein-sparing action of glucose would affect the plasma as well as tissue protein cannot be answered from the present data; it would require experiments in which both the plasma volume and the nitrogen excretion were measured.

The independence of the globulin fraction of the plasma during the intake of the protein-deficient diet in these experiments confirms previous clinical observations of Peters,[1] experimental findings by Weech and his co-workers,[4] as well as other experiments from this laboratory.[14, 15] In other words, removal of protein and fat intake affects only the albumin fraction of the plasma, and not the globulin.

Worthy of mention is the fact that in the present experiments and in others,[14] as first shown by Weech and associates,[4] the fall in albumin begins at once, and is progressive, initially at least, as long as protein is withheld from the diet. If there is such a thing as "deposit protein," or "reserve protein," it certainly fails to manifest itself under the conditions of these experiments. It would seem, therefore, that hypoproteinemia due to deficiencies in dietary protein cannot be prevented by body protein, or some other source of endogenous protein, which suggests that the concept of reserve protein may, perhaps, not apply to hypoproteinemia produced by dietary means alone.

SUMMARY

1. A pronounced (29 per cent) fall in the concentration of the albumin fraction of the plasma was observed in 5 dogs on a protein- and fat-deficient diet for three weeks; a similar but much smaller (14 per cent) fall occurred in 10 other dogs that were fasted completely. The globulin fraction remained relatively unchanged.

2. The less pronounced hypoalbuminemia in the animals completely fasted was accompanied by an increased red blood cell volume and was, therefore, due in part at least to dehydration; in a few instances of pronounced hemoconcentration the decline was masked or even replaced by a rise.

3. The observed hypoalbuminemia began with the onset of the experiment and was progressive. This indicates the absence of any store of reserve protein for the manufacture of serum albumin during a dietary induced deficiency.

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LABORATORY METHODS

GENERAL

SIMPLIFICATION OF FLUORESCENCE MICROSCOPY*

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THE fluorescence method of microscopy for the demonstration of *Mycobacterium tuberculosis* has recently attracted some attention in this country, but a more general testing and appraisal of the procedure is likely to be delayed because of a belief that the apparatus required is elaborate and complicated, or else expensive and difficult to obtain. Furthermore, commercial equipment already under test in some laboratories has met a most unfavorable reaction on the part of the workers who use it because the illumination is so dim that the tubercle bacilli can be seen only in an almost dark room, and at the expense of great eyestrain during protracted examination of films. This is totally unnecessary, but unless a more satisfactory form of light is devised the whole method may fall into disrepute as impractical. The principle is so simple, however, and the application so easy that the method may well be given the investigation it deserves if the technique can be reduced to a minimum no more difficult than ordinary microscopic examination.

The principle is elementary. It has been found that organisms of the acid-fast group, when stained by any one of a series of yellowish dyes, retain the stain after decolorization with acid alcohol, while bacteria not acid-fast lose the stain. The dyes used for the staining, including auramine-O, berberine sulfate, and thioflavine, are those which fluoresce when illuminated by ultraviolet light just off the end of the visible spectrum, the so-called "glass ultraviolet," which passes through ordinary glass with only moderate reduction in intensity. If a film stained and decolorized as indicated is illuminated with a powerful light which has passed through a deep blue filter of appropriate transmission band, the organisms which have retained the stain fluoresce a bright orange yellow, while the rest of the field is dazzlingly blue. An orange-yellow glass disc on the diaphragm of the ocular, selected for color so that it passes the fluorescent light of the bacilli and is opaque to the blue light of the field, will allow the stained bacteria to flare out on a dark background. The appearance is much like that seen with dark-field illumination, though the principle is totally different, and the fact that the organisms are self-luminous from their fluorescence and in sharp contrast to their surroundings makes them appear much larger than usual, so that they are easily seen with a high dry objective, as of 4 or 8 mm. focal length.

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The descriptions of the fluorescence method in the German and American literature are likely to give the impression that a rather special form of lamp is needed to give the necessary ultraviolet output.[1-6] Since, however, the light passes through the ordinary substage condenser of the microscope, perhaps fully 2 cm. in thickness, as well as through a condensing lens on the light source and through the common glass slide, the ultraviolet requirements plainly cannot be very exacting. Likewise, the insistence that a polished front-surface aluminum mirror must be used in place of the usual microscope mirror, in the belief that a silver surface absorbs the ultraviolet, is unfounded. A powerful, concentrated source of light, like a carbon arc or a 500-watt projection bulb, fitted with a condensing lens of about 7 cm. focal length with the blue filter in front of the condenser will supply an illumination bright enough to permit examination of films in a room darkened only by drawing the window shades.

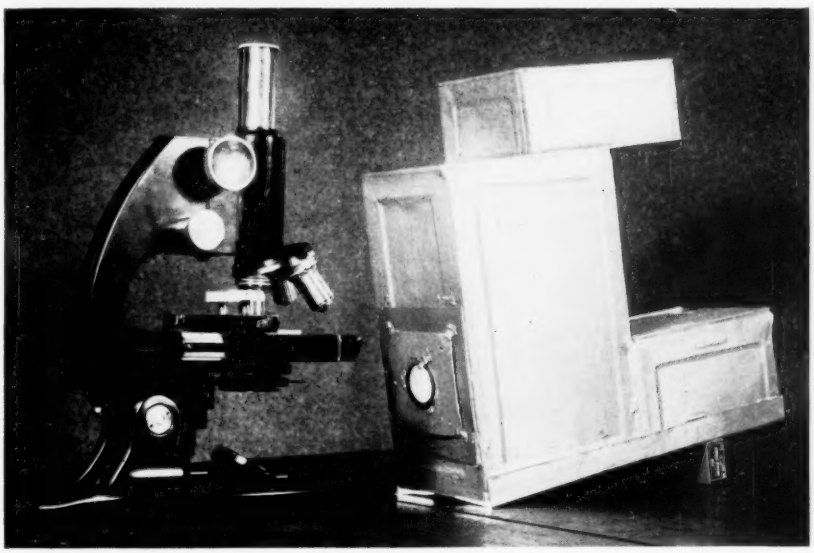

Fig. 1.

A satisfactory bulb for the purpose has been found in the 500-watt G. E. projection bulb operating on 110 to 115 volts. This is mounted in a ventilated sheet metal lamp box improvised in the laboratory. A condenser can be obtained from an old arc light, but a pair of ten-cent store reading lenses in tandem has proved equally useful. The proper relation of the lamp to the condensing lens is determined by trial and error, checking the effect of changes by the appearance of the field in the microscope. It has been found that the lamp should be set with the plane of the filament coils at an angle of about 30° to the optical axis so that the images of the coils will nearly overlap, as seen in the lighted field of the microscope. The housing should be large and provided with ample air inlet and outlet to dissipate the heat rapidly. It has also been found that the filter is better placed in front of the condensing lens rather than behind it. The beam of light should practically cover the mirror of the microscope, and its location is best seen when the mirror is half covered with a card during

adjustment. This arrangement calls for only an ordinary commercial bulb of relatively low cost, plus the application of a little ingenuity in making the housing, and dispenses with all special equipment, except the blue and yellow filters.* A substitute for the blue filter can be made with an ammoniacal solution of cupric sulfate contained in a flat-sided bottle, and adjusted to the proper depth of color by watching the microscope field as the solution is diluted.

For the benefit of those who have not had the opportunity to consult the literature which outlines the fluorescence technique, the procedure is described in its simple detail.

Heat-fixed films are stained in the cold for three minutes with a solution of auramine-0 (national aniline) made up as follows: auramine-0, 0.1 Gm., phenol absolute, 3 ml., and freshly distilled water, 97 ml. This solution should be kept in the dark, and made fresh every two weeks. It forms a slight precipitate without apparent loss of staining property. The film is rinsed in running water and decolorized for two minutes, and again for five minutes, with two applications of the solution: concentrated hydrochloric acid, 4 ml., sodium chloride, 4 Gm., 70 per cent alcohol, 1 liter. Decolorization may be continued much longer if desired, even for twenty-four hours. The slide is rinsed in running water, dried, and examined with the 4 mm. objective and a 10× ocular, or with an 8 mm. objective and a 20× ocular.

In films made from concentrates of sputum by sodium hydroxide digestion, practically no fluorescent material is seen, except the auramine-stained bacilli, so that a question often asked by persons seeing such a preparation for the first time will be, "Is this from a culture?" Direct films from sputum or spinal fluid, however, may contain many fluorescent inflammatory cells which hinder the examination. In such cases the suggestion of Herrmann may be followed, to counterstain for a few seconds with 0.1 per cent potassium permanganate solution followed by Loeffler's methylene blue, to make the cells so dark a blue that they become invisible.[2] In this laboratory concentrate films are examined routinely, so that no difficulty has arisen, except in the case of the spinal fluid of tuberculous meningitis, in which instance the counterstain has worked perfectly. Since a properly stained preparation will show nothing fluorescing except the bacilli, if any are present, it may be difficult to focus on the plane of the slide with no reference object in view. To meet this difficulty the suggestion has been made that a dot or streak of the auramine stain be put on one end of the slide just before it is examined, to give a reference plane for focusing.

In adjusting the illumination, it has been found convenient to pick up the light from the plane mirror with the low-power objective and center it properly before the high dry lens is swung into use. If organisms are abundant, they can usually be seen with the low power directly, and if the low- and high-power lenses are parfocal, the final adjustment is much simplified. The low-power field appears dark red, greenish, or almost black with any fluorescent particles showing as bright dots of light. The condenser should be at its top position, as lowering it gives a red or light-colored background. The aluminized mirror, as stated, is not necessary, but the microscope mirror and condenser should be kept reasonably clean.

*These may be obtained from the Spencer Lens Company.

The use of the immersion lens is unnecessary in routine examination of films, but it may be employed to check on the morphology of bacilli that appear atypical. Cedar oil, mineral oil, and sandalwood oil are not satisfactory as immersion media, since they all fluoresce and cast a haze over the field, but methyl salicylate (oil of wintergreen) is an excellent immersion oil with a high refractive index, and does not fluoresce. It can also be used as a mounting medium in the examination of tissue sections by the fluorescence method.

It has been noticed that an auramine-stained film will fade in the area exposed to the ultraviolet light if left in the field for about ten minutes. The fluorescence, of course, implies absorption of energy from the activating light, and apparently photochemical changes also take place. Likewise, stained films fade after the lapse of time, and the brightest preparations are those which have just been made, but restaining is satisfactory at any time. A Ziehl-Neelsen stain can be superimposed upon an auramine stain, but not vice versa.

Examination of fluorescence films stained with auramine-0 is considerably easier than similar examination of Ziehl-Neelsen films, because of the wide field covered by the dry objective and because the organisms are either seen or not seen without confusion with their surroundings, provided the illumination is bright enough to cause brilliant fluorescence. This proviso is the reason the use of such a high-power lamp is recommended. Probably at least 95 per cent of the light from the bulb is stopped in the filter, and the near ultraviolet output of a filament bulb is not great in any case, so that a large initial energy input is needed if enough active light is to reach the film to cause bright fluorescence. Fortunately economy is not an important factor in the choice of a light source, and the high-power lamp, ready to operate the moment it is plugged in, answers all practical requirements. Once the light has been put together and the adjustments of mirror and condenser completed, the system can be used indefinitely if no changes of position are made.

A photograph of the improvised light housing is shown to indicate its general manner of construction. Other designs of housing or other types of bulb may prove even more useful. It is hoped that simplification of the technique may stimulate further test of this perhaps valuable method of investigation.

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PRODUCTION OF EXPERIMENTAL HYPERTENSION AND THE INDIRECT DETERMINATION OF SYSTOLIC ARTERIAL PRESSURE IN RATS*

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THE assay of antipressor extracts of kidney requires the use of large numbers of hypertensive animals. Dogs have been extensively employed, but their disadvantage is the high cost of care and the resultant limitation of the number of observations. Rats are cheaper, but many investigators have had difficulty both in producing hypertension and in measuring their arterial pressure. The purpose of this communication is to record the experience we have had in the preparation and study of over 1,000 hypertensive rats.

PRODUCTION OF HYPERTENSION

Page (1939) described the use of silk and cellophane for eliciting hypertension by formation of a perinephric fibrocollagenous hull around the parenchyma of the kidneys. Thin China silk, organza silk, or even thin cotton batiste may be used. After anesthetizing the rat (males weighing 140 to 160 Gm. are most satisfactory) with seconal (sodium propyl-methyl-carbinyl allyl barbiturate, Lilly, 35 to 40 mg. per kilogram injected subcutaneously), the forelegs are tied down with the back of the animal against the operating board, while the hind legs are tied with the abdomen down, so twisting the animal in such a way as to give excellent exposure of the kidneys. The operator's hands and instruments are scrubbed, but aseptic technique is unnecessary. An incision is made parallel with the vertebral column, beginning in the costovertebral angle, and the kidney is delivered with the aid of chalazion forceps. The fat is stripped off, the silk is folded around the kidney and held in place by either a string around the pedicle or a purse-string ligature. The silk should be applied smoothly. The kidney is then replaced and the muscle and skin are closed with silk thread (No. A). Silk may be applied to both kidneys or only to one and its partner removed. Page, Patton, and Ogden (1941), and Williams, Harrison, and Grollman (1942) have modified this procedure by applying collodium instead of silk, but the latter investigators do not find it as satisfactory as silk.

We have also used the method employed by Drury (1938) for producing hypertension in rabbits, namely, constricting the main renal artery by a loop of silk thread. A wire approximately one-third the diameter of the normal artery

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(28 gauge wire is satisfactory for a 150 Gm. rat) is employed. A midline incision is made, the relative size of the renal artery is estimated with the help of a jeweler's loupe, and a wire is selected. The silk loop is placed around the wire and renal artery proximal to the suprarenal branch, and the wire is withdrawn. Since the left renal artery is the more accessible, this side is used when only one vessel is to be constricted. If the right artery is employed, the loop is placed proximal to the point at which the vessel runs dorsal to the inferior vena cava.

With either of these methods, if both kidneys are operated upon, the hypertension will usually be severe, and some will exhibit the malignant syndrome. If only one kidney has been treated, many rats develop satisfactory hypertension. If, however, arterial pressure has not risen after three weeks, the normal kidney may be removed or treated as was the other. When a silk loop has been employed in the initial operation and the pressure has not risen, it is desirable to inspect the kidney before deciding on the next procedure. If it is completely degenerated, or almost so, a second loop should be placed on the opposite renal artery. If, on the contrary, it is normal or even enlarged, but has the appearance of being congested, the partner had best be removed.

Hypertensive rats appear to need supplements to their diet in the form of brewer's yeast (2 per cent of the total diet, strain G, Anheuser-Busch) and liver extract (5 per cent of liver extract No. 343, Lilly). They seem more susceptible to lice and more frequently develop dietary deficiencies than normal rats. In our hands we consider the technique satisfactory if 50 per cent of the animals develop hypertension.

It is of interest that if a rise of arterial pressure to 200 mm. Hg or more develops within a few days of operation, death usually occurs unless a fall in pressure is induced by injection of kidney extract. Under these circumstances they may recover and again become hypertensive on discontinuing the extract, but usually less intensively than before treatment. The time of appearance of hypertension is often very variable, from several days to several months being the rule.

DETERMINATION OF SYSTOLIC ARTERIAL PRESSURE IN RATS

The method of Williams, Harrison, and Grollman (1939) has been used. We have found it very satisfactory if a few modifications are introduced. In essence the method consists in determining the pressure at which blood begins to flow into the tail after compression by a cuff, as indicated by a plethysmograph.

Apparatus.—The sphygmomanometer cuff (*G*) in the diagram of Williams, Harrison, and Grollman (1939) was reduced to 2.6 cm. length because a greater length of tail was left for the plethysmograph, and correspondingly the readings were sharper. The tubing should be sufficiently loose so that not more than 10 mm. Hg pressure are required to collapse it. The plethysmograph may be made more sensitive by tying off the rubber sleeve within it at the distal end instead of leaving it open as in the diagram, but we prefer the original method because

the movements of the animal are not unduly magnified. The water in the plethysmograph is warmed by means of an electric light bulb placed close to the syringe *R*. The bore of the rubber tubing leading from the plethysmograph to the water manometer may be as small as $\frac{3}{32}$ inch in diameter, and the length need not be inconveniently shortened if the wall is thick. The water manometer

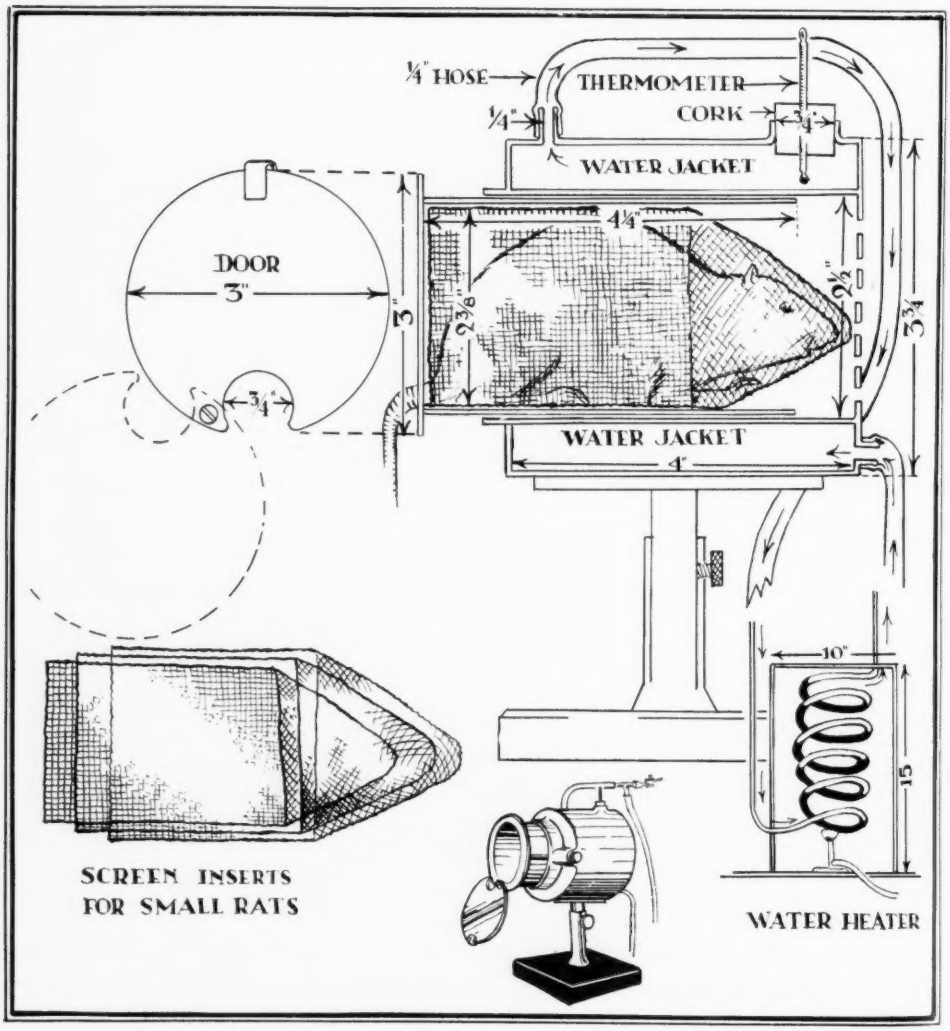

Fig. 1.—Modifications of Williams, Harrison, and Grollman's method for systolic blood pressure in rats.

is easier to read if a little safranine is dissolved in the water filling it. It is important to change the fluid daily to prevent growth of mold which may clog the tubing. Cautery set double rubber bags* seem more convenient than the ordinary sphygmomanometer bulb for reduction of pressure within the system. Instead of using these bulbs for inflating the sphygmomanometer cuff (*G*), air from the laboratory line is employed.

*Double blast cautery bags may be secured from the Davol Rubber Co., Providence, R. I.

The holder (*A*) for the rat has been adapted (Fig. 1) from that of Chen and Rose (1939) and has been found greatly to facilitate handling of the rats. It is surrounded by a water jacket connected with a copper coil placed in a can which is insulated with asbestos and heated with a micro gas burner. Screen wire adapters (Fig. 1) are used to prevent excess movement by fitting the rat more snugly in the holder.

PROCEDURE

The rat is warmed for four to five minutes in a box kept at 55° to 58° C., placed in the holder (warmed to 40° to 42° C.), and the tail is inserted into the plethysmograph (warmed to 40° C.). Gentle pressure is exerted on the tail by pressing the plunger of the syringe down, the cuff is inflated above the probable systolic pressure, and the two-way cock is turned to connect the pethysmograph with the water manometer. Air is allowed to escape until the water in the manometer begins to rise. If the escape of air is stopped at this point, the mercury in the sphygmomanometer will return to the point at which the water in the manometer began to rise. This return is due to the elastic recoil of the blast bulb. Because of the blast bulb the escape of air can be more rapid and the readings more accurate.

The nearer one is to systolic pressure the slower will be the rise of water in the manometer. A continued rise demonstrates that the change is due to blood entering the tail rather than to the movement of the rat. When the air pressure is lowered to less than venous pressure in the tail, the water in the manometer should fall. Four or five readings are taken on each animal.

COMMENT

Comparison of the shorter sphygmomanometer cuff with the longer one shows that readings are sharper and more rapidly taken. Overheating the rats, as evidenced by red ears and paws, overactivity, and sweating, must be avoided. They may suffer a heat stroke and die. By this method of blood pressure determination more uniform and *higher* systolic pressures were obtained on properly warmed animals than on unwarmed or partly warmed ones. Preheating the rats is necessary, as Williams, Harrison, and Grollman state. We find the shorter time at a higher temperature, as suggested by them, somewhat more effective than five to ten minutes at 40° to 42° C., and we also believe that warming the rat holder is essential. The change in the design of the holder has the very considerable advantage that the rat may be introduced more easily and without excitement. Excited, frightened animals obviously give false values; it is probably not well recognized that defecation while in the holder may raise the pressure 30 mm. Hg, although occasionally a fall will occur. Unusual sounds, especially strange voices, usually affect the readings. Handling the rats by their tails should be scrupulously avoided, as well as giving intramuscular or subcutaneous injections in this region.

The average systolic pressure taken daily for five weeks in five of our normal rats was within the limits of 100 to 120 mm. Lower and much more irregular readings are obtained if the animals and the animal holders are not

warmed. Single readings with the warmed rats were found to correspond with the *average* readings in about one-half of the trials. The other half varied from 20 mm. Hg to occasionally as much as 100 mm. Hg from the average. These results caution against accepting a single reading.

For testing the antipressor potency of kidney extracts we find animals with arterial pressure about 200 mm. Hg most satisfactory. The normal fluctuations of such animals is about 20 mm. Hg; hence a satisfactory extract, when given daily, must reduce the pressure below 150 mm. Hg. After discontinuing extract, the return to the initial blood pressure level usually takes more than a day. At least three hypertensive rats are used for each assay. It is important that the personnel dealing with these animals be sympathetic toward them, and that injections of extracts not be made at or near the time blood pressure readings are taken. If the latter precaution is observed, it will be found much easier to train the animals.

SUMMARY

1. Silk perinephritis and constriction of the renal artery by a silk thread both elicit arterial hypertension in rats of a degree sufficient for assay of renal antipressor extracts. The preparation of hypertensive rats by these methods is described.

2. The method of Williams, Harrison, and Grollman for measurement of systolic blood pressure has been modified to increase its effectiveness.

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A QUANTITATIVE MICROPIPETTE FOR INTRANASAL INOCULATION OF MICE WITH VIRUS SUSPENSIONS

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IT IS standard practice in most laboratories engaged in research on viruses to employ small hypodermic syringes and needles for the intranasal inoculation of mice. When large numbers of animals are being handled, as in our current influenza investigations, we have found that this technique not only requires a very considerable outlay for syringes, but is tiring and somewhat cumbersome in practice.

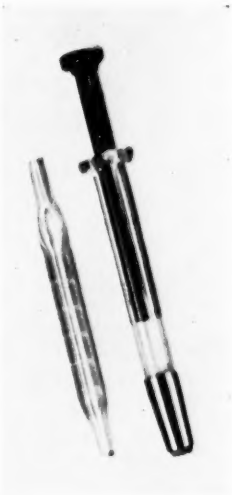

Fig. 1.—Micropipette (left) and syringe fitted with adapter (right).

The apparatus shown in Figs. 1 and 2 was developed to replace the syringe-needle assembly, and has been used with satisfaction during the past several months. The micropipette (left, Fig. 1) is 3¾ inches long and is graduated to deliver five aliquots of 0.05 ml. each. The top of the pipette contains a safety bulb beyond which it tapers to fit in the adapter of the plunger unit. The latter consists of an ungraduated syringe with colored ground-in piston; it is used to generate suction for filling the micropipette and to regulate the delivery of virus suspensions from it.

*The Unit Personnel consists of Commander A. P. Krueger; Lieutenan's (j.g.) W. P. Chesbro, L. E. Rosenberg, and N. S. West; Ensigns A. S. Browne, O. J. Golub, and J. R. Mathews; CPhM, I. L. Shechmeister and T. P. Sislock; PhM 1c, W. L. Axelrod; PhM 2c, E. R. Chisholm and G. B. Saviers; and PhM 3c, H. R. Burkhead and C. R. Webb, Jr.

The opinions advanced in this paper are those of the writers and do not represent the official views of the Navy Department.

Under working conditions the plunger unit is mounted rigidly in a burette clamp, and micropipettes are fitted into the adapter as needed. Usually a fairly large series of pipettes will be employed in succession, using a clean one for each

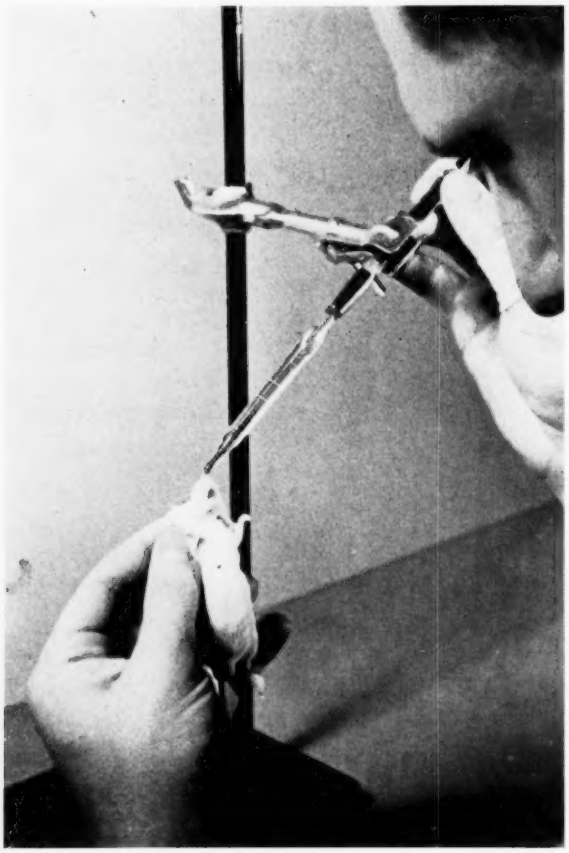

Fig. 2.—Micropipette assembly in use for conducting intranasal inoculations.

virus-containing suspension to be inoculated. The pipettes cost about one-half as much as a regulation 0.25 ml. hypodermic syringe, and since only one or two plunger units per operator are required for a day's work, the total cost for equipping a laboratory is very reasonable.

This equipment is available through Mr. C. E. Camenson, The Micro-Chemical Specialties Company, 2112 Berkeley Way, Berkeley, Calif.

SODIUM PENTOTHAL ANESTHESIA FOR ABDOMINAL SURGERY IN EXPERIMENTAL ANIMALS*

Induction by Intravenous Injection and Maintenance by Intermittent Intraperitoneal Drip

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SHORT duration between administration and excretion is a desirable attribute of any anesthetic because it permits more immediate control of the anesthetic level. The adage "better to repeat than to repent" has been impressed upon many surgeons and anesthetists working with various long-action anesthetic agents.

While performing certain experimental abdominal procedures in dogs and rabbits, we developed a maintenance technique with sodium pentothal† which permitted us to dispense with the services of an anesthetist for the entire procedure. If we worked in pairs, the assistant induced the anesthesia by intravenous injection while the operator remained attired in sterile gown and gloves. If we worked alone, the operator induced the anesthesia by intravenous injection while wearing an extra pair of rubber gloves, which were removed before starting the abdominal operation. The instrument tray contained a sterile syringe with a hypodermic needle into which the operator drew sodium pentothal for intraperitoneal administration during the course of the operation.

For rabbits we used 1.25 and 1 per cent solutions of sodium pentothal. The latter was more satisfactory. The animals weighed approximately 4 pounds. They were placed in a box with the head protruding, and 2 to 3 c.c. of 1 per cent solution were administered in the lateral marginal vein of the ear. With this amount, corneal reflexes still remain. Five cubic centimeters of 1 per cent solution are lethal. The animals were then strapped on the operating table and the procedure was started. If a minimal induction dose was used, the animal might be restless during the opening of the peritoneum. A few drops of 1 per cent solution administered from the syringe on the tray would stop this. After the peritoneum was opened the entire length of the incision, little difficulty was encountered. Maintenance of anesthesia was done by dropping intermittently a small amount of sodium pentothal into the open abdomen, guided by restlessness of the animal and the character of its respirations. Shallowness of respiratory exchange indicated too great depth. An average

*From the Foundation for Clinical and Surgical Research, Dr. John O. Bower, Director.
†A product of the Abbott Laboratories.
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anesthesia was performed for wedge gastric resection wherein 2 c.c. of 1 per cent solution were given intravenously for induction, and 1.4 c.c. by intraperitoneal drip for maintenance. The operative procedure lasted one-half hour. More than 50 rabbits have been operated on with the above technique.

For dogs we used 2 and 2.5 per cent solutions. The animals were strapped on the operating table, and the induction dose was given into a superficial vein of the forepaw or the hind leg. The amount necessary varied with the size and vigor of the animal. Morphine and atropine preoperatively were of great advantage. Dogs weighing less than 20 pounds received morphine sulfate gr. $\frac{1}{8}$ and atropine sulfate gr. $\frac{1}{150}$; those weighing more than 20 pounds received morphine sulfate gr. $\frac{1}{4}$ and atropine sulfate gr. $\frac{1}{100}$. This preoperative sedation was given hypodermically forty-five minutes before. Little difficulty was found in opening the peritoneum. Anesthesia was maintained by intermittent intraperitoneal drip. Anesthetic level was determined by respirations and restlessness, and the status of corneal reflex was obtained by touching the cornea with a piece of gauze which was then discarded. A representative anesthesia was performed in a 60 pound dog, to which 14 c.c. of 2.5 per cent solution were given intravenously for induction and 12 c.c. were given intraperitoneally for maintenance during a procedure on the biliary ducts, lasting one hour. More than 25 dogs have been operated upon with this anesthetic technique.

2008 Walnut Street

THE SEDIMENTATION RATE IN HODGKIN'S DISEASE*

Bowman Wise, M.D., Durham, N. C.

IT IS generally recognized that the erythrocyte sedimentation rate usually is significantly elevated in Hodgkin's disease, but that it is of no aid in the differential diagnosis of this disease. However, the relationship of the sedimentation rate to phases of activity, or relative quiescence, of the disease has not been studied. The present study was undertaken to determine whether the sedimentation rate might be of aid in determining the state of activity of the Hodgkin's disease process, thereby furnishing indications for treatment, especially during intervals in which the patient is symptom free and has no objective evidence of activity of the disease.

MATERIAL AND METHOD OF STUDY

The sedimentation rate of the red blood corpuscles has been determined by the Wintrobe method.[1]

The data reported here have been accumulated from patients in Duke Hospital in whom the diagnosis of Hodgkin's disease has been established histo-

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pathologically. Sedimentation rates have been determined at the time of the initial visit and, whenever possible, at intervals throughout the period of observation. Because patients frequently leave the hospital or out-patient clinic promptly upon the completion of roentgen therapy, it has not been possible to make observations of the immediate effect of such therapy on the sedimentation rate.

RESULTS

Single determinations of the sedimentation rate were made on 17 patients suffering from Hodgkin's disease, the results of which are given in Table I. They show a significant elevation of the sedimentation rate in Hodgkin's disease and an absence of any correlation with the duration of the disease or therapy. In all these patients, because of the existence of fever, obvious progressive lymph node enlargement, or evidence of progressive visceral or osseous involvement, the disease process was considered to be active at the time the sedimentation rates were determined.

Thirteen patients afforded an opportunity to make repeated determinations of the sedimentation rate during the course of the disease. The case histories of the majority of these patients have been reported in detail elsewhere[2] and are summarized briefly here in the numerical sequence in which the patients came under observation.

TABLE I

BLOOD SEDIMENTATION RATES OF 17 PATIENTS SUFFERING FROM HODGKIN'S DISEASE

PATIENT	RACE	SEX	AGE	CORRECTED SEDIMENTATION RATE (MM./HR.)	DURATION OF DISEASE BY HISTORY	PREVIOUS THERAPY
J. O.	W	M	20	43	2½ years	Roentgen therapy
L. S.	W	F	44	44	2 years	Roentgen therapy
V. W.	W	F	26	40	8 to 9 years	Roentgen therapy
M. H.	W	F	24	29	20 months	None
S. S.	W	M	38	34	21 months	Roentgen therapy
E. W.	W	M	28	39	8 years	Roentgen therapy
V. H.	W	F	21	42	6 months	None
P. T.	W	F	29	39	22 months	Sulfapyridine and roentgen therapy
C. S.	W	M	38	37	4 years	Roentgen therapy
J. C.	W	M	30	32	13 months	Roentgen therapy
S. M.	W	F	43	38	3 months	Roentgen therapy
E. M.	W	M	26	32	9 months	None
P. C.	W	F	25	46	8 months	None
K. G.	W	M	17	40	5 years	Roentgen therapy
F. T.	W	M	30	40	2 months	None
S. G.	W	M	38	32	8 months	Roentgen therapy
W. D.	W	M	31	25	2 years	Roentgen therapy

Case 1 is of particular interest because of the demonstration of normal or nearly normal sedimentation rates during periods in which there was no evidence of activity of the disease. In this patient elevation of the sedimentation rate from a previous normal level preceded by six weeks any definite evidence of recurrent activity of the disease process.

CASE REPORTS

CASE 1.—R. P., a 24-year-old white man, entered the hospital on Oct. 26, 1938, complaining of right cervical lymph node enlargement of one year's duration, which had been treated by excision and superficial radium therapy. At the time of admission a lymph node, 3 to 4 cm. in diameter, and several smaller nodes were palpable in the right cervical region. *Brucella suis* was isolated from a right cervical lymph node which had the characteristic histopathologic appearance of Hodgkin's disease.

DATE	CORRECTED SEDIMENTATION RATE MM./HR.	SUBSEQUENT COURSE
10/27/38	5	Regression of right cervical lymph node enlargement under treatment with sulfanilamide and serum from a person considered immune to brucella infection
7/31/39	4	No evidence of activity of the Hodgkin's disease process
9/23/39	8	Questionable slight recurrent enlargement of right cervical lymph nodes following acute pharyngitis two weeks previously
11/27/39	20	No evidence of activity
1/29/40	34	Recurrent right cervical lymph node enlargement regressing slightly under sulfanilamide and roentgen therapy
4/15/40	34	Condition unchanged
6/10/40	25	Cough of two weeks' duration; mediastinal mass demonstrable in chest roentgenogram; marked diminution of cough during treatment with sulfathiazole, but size of mass unchanged; roentgen therapy instituted to mediastinum
8/ 6/40	34	Chills and fever of three weeks' duration; complete atelectasis of left lung from pressure of mediastinal mass; roentgen therapy
10/ 8/40	36	Fever; weight loss; persistent atelectasis of left lung; liver and spleen palpable
10/ 8/40	34	Condition unchanged under further roentgen therapy
12/ 2/40	15	Fever, marked weight loss and anemia; reexpansion of lung; liver and spleen increase in size; questionable infiltration of lumbar spine; no improvement under roentgen therapy; death on Feb. 7, 1941

Cases 2, 7, and 11 show a tendency of the elevated sedimentation rate to remain at a constant level during a period of months, even though temporary improvement in the patient's condition or relative inactivity of the disease may be apparent.

CASE 2.—M. W., a 5-year-old negro boy, was brought to the hospital on Nov. 16, 1938, with an enlargement of his neck of ten months' duration. It was not possible to admit the patient to the hospital until Jan. 26, 1939, at which time examination revealed marked enlargement of the left cervical and left axillary and inguinal nodes. A chest roentgenogram showed a mediastinal mass. Lymph node biopsy confirmed the clinical diagnosis of Hodgkin's disease. Under treatment with sulfapyridine there was a marked decrease in the size of the enlarged superficial lymph nodes, and roentgenographic examination showed diminution in the size of the mediastinal mass. The patient was discharged from the hospital on March 3, 1939. In May, 1939, he was readmitted to the hospital with an enlargement of the left inguinal and femoral lymph nodes, but there was no recurrent enlargement of other superficial lymph nodes, and a chest roentgenogram showed complete absence of the mediastinal

mass noted before. His temperature reached 39° C. *Brucella melitensis* was isolated from a blood culture. Following treatment with sulfapyridine and bovine antimelitensis serum, the patient became afebrile, and the enlarged lymph nodes had decreased in size by the time of discharge on June 4, 1939. The patient's subsequent course is tabulated below:

DATE	CORRECTED SEDIMENTATION RATE MM./HR.	SUBSEQUENT COURSE
9/ 8/39	28	Fever and recurrent left cervical, axillary and inguinal lymph node enlargement of one week's duration, subsiding under treatment with sulfapyridine
2/ 5/40	28	History of intermittent brief episodes of fever; transient left cervical enlargement one week previously
6/24/40	30	Condition unchanged; no demonstrable recurrence of lymph node enlargement
12/12/40	21	Intermittent enlargement of superficial lymph nodes accompanied by fever of one month's duration; spleen palpable
6/13/41	22	Marked enlargement of cervical, axillary, and inguinal lymph
6/23/41	26	nodes; fever of one week's duration; spleen and liver
6/27/41	15	palpable; prompt regression of lymph node enlargement under sulfathiazole

CASE 7.—M. T., a 31-year-old white woman, experienced the onset of persistent generalized itching in November, 1936, along with enlargement of left cervical and left axillary nodes which fluctuated in size, but there had been recurrent enlargement of these nodes for two months before admission to the hospital on March 8, 1939. Lymph node biopsy confirmed the diagnosis of Hodgkin's disease. Her subsequent course is given below:

DATE	CORRECTED SEDIMENTATION RATE MM./HR.	SUBSEQUENT COURSE
3/ 8/39	38	Left cervical and axillary lymph node enlargement; severe pruritus; lessened pruritus and decrease in the size of the enlarged lymph nodes under treatment with sulfapyridine
5/ 7/39	38	Recurrence of lymph node enlargement with enlargement of right cervical and axillary nodes, and small areas of density shown in the right lung by roentgenogram; stomatitis; fever; improvement under sulfapyridine
7/18/39	28	Recurrent superficial lymph node enlargement; cough and extensive mediastinal and hilar enlargement demonstrable roentgenographically; intensified pruritus; improvement under roentgen therapy
9/11/39	44	Further roentgen therapy; cough relieved; pruritus unchanged
10/18/39	43	Slight enlargement of left cervical lymph nodes; increasing weakness
11/23/39	44	Fever; extension of pulmonary parenchymal involvement; progressive weakness, weight loss, and anemia; further roentgen therapy
2/12/40	37	Afebrile; further superficial lymph node enlargement, including inguinal nodes; decrease in size of pulmonary lesions by roentgen examination
3/14/40	42	Fever and progressive cachexia; vague abdominal discomfort; further roentgen therapy
9/12/40	18	Continued fever and progressive cachexia terminating in death on Sept. 20, 1940

CASE 11.—A. A., a 40-year-old woman, entered the hospital on June 27, 1939, complaining of weakness, loss of weight, and enlargement of the neck of seven months' duration. Above the suprasternal notch and in the right cervical region were numerous enlarged lymph nodes constituting a mass 5 by 4 cm. The spleen was palpable. A chest roentgenogram showed a large mediastinal mass. Lymph node biopsy confirmed the clinical impression of Hodgkin's disease, and from the biopsied node and from the blood *Brucella suis* was isolated. Her subsequent history is summarized below:

DATE	CORRECTED SEDIMENTATION RATE MM./HR.	SUBSEQUENT COURSE
6/27/39	34	Moderate decrease in cervical and mediastinal lymph node enlargement under roentgen therapy
9/ 8/39	34	Weight gain of 11 pounds in interval; no recurrent lymph node enlargement; marked fatigability
12/22/39	34	Slight recurrent enlargement of cervical nodes; enlargement of left inguinal nodes; roentgen therapy

Cases 6, 17, and 18 show a definite fall in the sedimentation rate following sulfonamide and/or roentgen therapy.

CASE 6.—C. I., a 40-year-old white woman, in June, 1938, noted gradual enlargement of the superficial lymph nodes accompanied by fever and marked fatigability. In January, 1939, mediastinal enlargement was demonstrable, and the liver and spleen were palpable. Lymph node biopsy established the diagnosis of Hodgkin's disease. Under roentgen therapy her fever subsided, the lymph node enlargement disappeared, and the liver and spleen were no longer palpable. Her subsequent course as is known is given below:

DATE	CORRECTED SEDIMENTATION RATE MM./HR.	SUBSEQUENT COURSE
3/ 1/39	26	Initial visit two weeks after completion of roentgen therapy
6/ 6/39	36	Fever and slight enlargement of a right cervical node; spleen palpable; *Brucella melitensis* isolated from blood cultures. Prompt subsidence of symptoms and splenic enlargement under treatment with sulfapyridine and bovine antimelitensis serum
7/20/39	19	Entirely symptom free

CASE 17.—G. R., a 16-year-old white boy, entered the hospital on March 8, 1940, complaining of superficial lymph node enlargement of two years' duration. Lymph node biopsy in August, 1939, established the diagnosis of Hodgkin's disease, and he was subsequently given roentgen therapy, with reduction in the size of the enlarged nodes. At the time of admission to the hospital there were daily elevations of temperature to 39° C., a few small firm lymph nodes were generally palpable, and the spleen, which was firm and nontender, extended 10 cm. below the costal margin. A chest roentgenogram was not remarkable.

DATE	CORRECTED SEDIMENTATION RATE MM./HR.	SUBSEQUENT COURSE
3/4/30	18	Initial visit
3/8/40	18	Temperature fell under sulfathiazole therapy, but the patient
4/5/40	7	did not become afebrile until roentgen therapy was instituted to splenic area; spleen ceased to be palpable

CASE 18.—J. Q., a 23-year-old white man, was referred to the hospital on April 20, 1940, for study. His illness began in December, 1934, with generalized pruritus, which persisted until the summer of 1937. In May, 1937, a slight enlargement of the cervical and axillary lymph nodes was noted, and the histopathologic findings in an excised node were typical of Hodgkin's disease. A chest roentgenogram taken at this time showed hilar enlargement. Roentgen therapy was instituted in August, 1937, since which time the patient had been almost symptom free. In September, 1939, a few small nodes were noted in the axillae. At the time of his visit to the hospital he felt entirely well, but he had noted an elevation of temperature to 99.4° F. each evening of the preceding week. Physical examination revealed two very small palpable right axillary nodes. His subsequent course during the period of observation is given below:

DATE	CORRECTED SEDIMENTATION RATE MM./HR.	SUBSEQUENT COURSE
4/20/40	47	Initial visit
6/ 7/40	30	Condition unchanged. Slight daily elevation of temperature continued
6/17/40	14	Patient received sulfathiazole from 6/11/40 to 6/26/40
6/19/40	11	

DISCUSSION

The finding of a normal or low erythrocyte sedimentation rate during a period of apparent quiescence of the Hodgkin's disease, suggests that this procedure may have a distinct usefulness in following the course of the Hodgkin's disease process. In particular, the finding of an elevated sedimentation rate may aid in deciding when further therapy is advisable.

It has not been possible as yet in this study to determine the immediate effect of roentgen therapy upon the sedimentation rate in Hodgkin's disease. Patients in whom the progress of the disease process has been relatively constant and rapid, and who from time to time have received roentgen therapy, have maintained elevated sedimentation rates. However, these determinations of the sedimentation rate have not been made at sufficiently close intervals to furnish information regarding the effect of roentgen therapy during the period when its effect is clinically most obvious.

In patients suffering from Hodgkin's disease, treatment with sulfonamide drugs has frequently been followed by regression of lymph node enlargement, disappearance of fever, and improvement in the patient's general condition. Although the number of observations is few, a fall in the sedimentation rate has attended the good results of sulfonamide therapy. Only further investigation and experience can determine the proper place of the sulfonamide drugs in the treatment of Hodgkin's disease, but repetition of the sedimentation rate at frequent intervals during the treatment period, regardless of the type of therapy employed, may be expected to aid in the decision as to the amount and extent of treatment advisable.

Attention should be called to a fall in the sedimentation rate exhibited by two patients of this group, Cases 1 and 7, during the terminal stage of the disease. Wintrobe has stated that the sedimentation rate may be normal, or fall toward normal, in the markedly cachectic stage of tuberculosis, and that corrections of

the sedimentation rate in the presence of severe anemia may be misleading.[3] Both patients, mentioned above, exhibited severe anemia and cachexia, to which the fall of the sedimentation rates seems attributable.

SUMMARY

The sedimentation rate has been studied during the course of Hodgkin's disease. Although usually elevated in this disease, the sedimentation rate may be normal during periods of relative inactivity of the disease process. The usefulness of the sedimentation rate in following the course of Hodgkin's disease and as a guide in therapy is suggested.

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A MERCURY VACUUM RELEASE*

For Ultrafiltration and Other Evacuated Systems

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IN CONNECTION with many laboratory experiments it is desirable to maintain a vacuum of a given level. While a simple mercury trap is adequate when the vacuum desired is only a few millimeters of mercury, for a higher vacuum, bubbling and loss of mercury is troublesome unless a large diameter tube is used, in which event a great deal of mercury is required. The device we describe is easily constructed, and is capable of maintaining the vacuum in a given system with a variation of only a few millimeters.

A 120 mm. T-tube is connected to the upper end of a 50 c.c. burette; through this, held by a piece of pressure tubing, is run a length of 5 mm. glass tubing, of such dimensions that when bent the upper end extends about 2 inches below the mouth of the burette, and the lower end about 1 inch above the tip. By a heavy clamp, not shown in the diagram, placed near the upper end of the burette, the whole device is suspended in a bottle containing mercury. When connected to the system to be held at a given vacuum, the mercury rises in both tubes. When the level of the mercury drops below the tip of the glass tube, the mercury in this tube is drawn over, returning through the burette to the bottle.

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and allowing air to enter the system. By adjusting the depth which the tubes penetrate into the mercury, in conjunction with a manometer, the device may be set so that air enters the system at any given vacuum level, and is by-passed to the pump, making certain that the vacuum does not exceed the desired level.

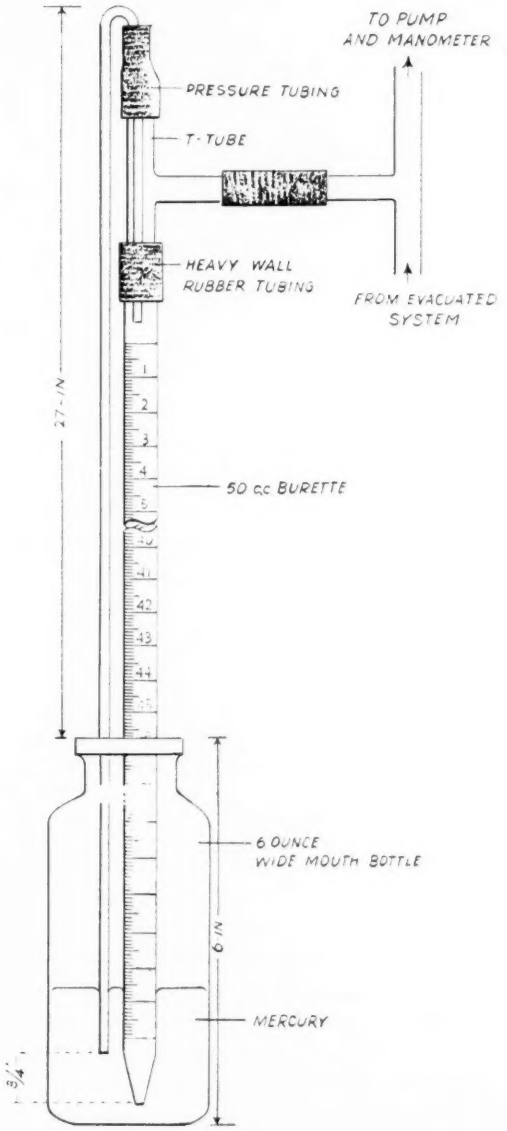

Fig. 1.

Variation in the internal pressure in the evacuated system may be minimized by drawing out the lower end of the tube to a small tip.

We have used this for maintaining vacua of from 15 to 360 mm. of mercury and have found it satisfactory for ultrafiltration and for holding animals at varying fractions of atmospheric pressure.

CHEMICAL

EFFECT OF STANDING ON CHOLESTEROL AND CHOLESTEROL ESTER VALUES IN HUMAN BLOOD*

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STUDIES on cholesterolysis, cholesterogenesis,† cholesterol ester hydrolysis, and esterification of the different parts of human blood (blood serum, blood plasma, whole blood) have presented varied and conflicting results.

Schultz,[1] Thannhauser,[2] Bloor and Knudson,[3] found no change in the cholesterol values on incubation of human blood while Cytronberg[4] and Sperry[5] report marked hydrolysis and marked esterification. Schube[6] states that blood cholesterol may change in vivo from week to week from 0 to 73 mg. He made a study[7] of 21 normal bloods and showed that after different time intervals and at different temperatures, there was a variation from 15 points above to 49 points below the original cholesterol values.

Inasmuch as the per cent of cholesterol esters in the total blood cholesterol is of diagnostic significance in cases of liver damage and jaundice,[3, 8-12] the question arose in our laboratory as to whether it was safe to allow the blood to stand any length of time before analyzing it for cholesterol and cholesterol esters, or whether these determinations should be made immediately after the blood was drawn.

To answer these questions, a comparative study of cholesterolysis, cholesterogenesis, and cholesterol ester hydrolysis and esterification on (a) oxalated blood, (b) plasma from oxalated blood, and (c) blood serum was made.

PROCEDURE

The bloods were selected at random from those of patients and blood donors‡ in the Cook County Hospital. Duplicate determinations for both total cholesterol and cholesterol esters were made immediately after the blood was drawn and after the specimens had stood for twenty-four hours.

The plasma and serum were separated from the cells by centrifugation and placed in cork-stoppered containers. The whole oxalated blood was kept in stoppered tubes. For the "immediate" analyses, aliquots were taken soon after the bloods were drawn, and alcohol ether filtrates were made at once (as directed below) and set aside in glass-stoppered bottles until the twenty-four-hour filtrates were ready. For the "twenty-four-hour" analyses the specimens were tightly stoppered and allowed to stand for twenty-four hours at room temperature or in the incubator at 37° C.; aliquots were then taken and alcohol-

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†"Cholesterolysis, cholesterogenesis" used in the same sense as the terms "glycolysis and glycogenesis."

‡Blood from normal donors was obtained through the courtesy of Dr. E. H. Schirmer, Director, Blood Bank, Cook County Hospital.

ether filtrates were made. Total cholesterol and cholesterol ester determinations were made simultaneously to rule out additional time factors.

Total Cholesterol (Bloor,[13] Sachett[14] method slightly modified).—Three cubic centimeters of oxalated blood, plasma, or serum are transferred into a 100 c.c. volumetric flask, using an Oswald-Folin pipette;* 3:1 alcohol-ether mixture is added through a funnel while agitating the flask.† Make up to volume, mix well, and filter. If the filtrate is tinted yellow (icteric blood), shake with Lloyd's reagent and refilter. Use aliquots of the clear filtrate for duplicate determinations‡ of cholesterol or cholesterol esters.

TABLE I

TOTAL CHOLESTEROL (MG./100 C.C.)

(RESULTS OF FIRST 20 CASES FROM A SERIES OF 78* CASES)

WHOLE BLOOD			PLASMA			SERUM		
IMMEDIATE VALUES	24-HOUR VALUES	DIFFERENCE	IMMEDIATE VALUES	24-HOUR VALUES	DIFFERENCE	IMMEDIATE VALUES	24-HOUR VALUES	DIFFERENCE
184	183	−1.0	115	120	+5.0	172	171	−1.0
163.5	157.5	−6.0	193	197.5	+4.5	168	168	0.0
172.5	170	−2.5	148.5	147	−1.5	279	279	0.0
170.5	168.5	−2.0	131.5	128.5	−3.0	241	240	−1.0
185.5	186	+0.5	185.5	186.5	+1.0	243	239.5	−3.5
151.5	150.5	−1.0	186	186.5	+0.5	202	198.5	−3.5
143	142	−1.0	126.5	125.5	−1.0	305.5	310	+4.5
190	190	0.0	225	226	+1.0	266	267	+1.0
154.5	155.5	+1.0	176	177	+1.0	206	204.5	−1.5
170.5	170.5	0.0	159.5	157	−2.5	181.5	177	−4.5
200	199	−1.0	181.5	182.5	+1.0	223.5	219	−4.5
150	146	−4.0	334	331	−3.0	213	209	−4.0
247	244	−3.0	155.5	155	−0.5	256	254	−2.0
214.5	212	−2.5	212.5	210	−2.5	147.5	149.5	+2.0
152	152.5	+0.5	195	195.5	+0.5	207.5	209	+1.5
148.5	146	−2.5	147	148	+1.0	157.5	156.5	−1.0
222.5	220	−2.5	168.5	169	+0.5	193	196	+3.0
250	250	0.0	213	213	0.0	250	252.5	+2.5
220	218.5	−1.5	151	152.5	+1.5	240.5	242.5	+2.0
218	213	−5.0	168	170.5	+2.5	169	167	−2.0

*Complete protocols available on request.

Cholesterol Esters.—Cholesterol is precipitated from the filtrate with digitonin. A quantitative separation of the cholesterol esters from the cholesterol digitonide is made by extraction with petroleum ether (b.p. 60° C.) as follows:

Add 15 c.c. of petroleum ether to the residue from 25 c.c. of evaporated filtrate. The mixture is thoroughly scraped from the sides and bottom of the beaker and triturated. Cover the beaker with a watch glass and boil on a water bath over an electric plate, rotating constantly to avoid bumping, until about one-half of the liquid has evaporated. Dry the beaker on the outside, and when boiling ceases, decant the solution with the aid of a stirring rod through a small funnel plugged with a small piece of cotton. The treatment with petroleum ether is repeated three or four times in order to insure quantitative extraction of the cholesterol esters. *The filtrate must be absolutely clear.* Any trace of turbidity will give high results. Evaporate the combined extracts to dryness, and proceed as under cholesterol with chloroform extraction.

*Twenty milligrams of potassium oxalate for 10 c.c. of blood.

†A fine granular precipitate is obtained, no lumps.

‡Not more than two or three sets of duplicates were set up at any one time.

Using the foregoing technique, we were always able to get like results with duplicate and triplicate samples.

Table I shows the difference between the cholesterol values of whole blood, serum, or plasma tested immediately after drawing, and again in twenty-four hours. This difference is so slight that it is of the same magnitude as the limits of experimental error. This indicates, contrary to the contention of Schube,[7] that the total cholesterol does not change significantly, if at all, when oxalated whole blood, plasma, or serum is allowed to stand at room temperature for twenty-four hours. It appears, therefore, that a specimen for total cholesterol determination may safely be analyzed within twenty-four hours.

On the other hand, Table II shows that the values of cholesterol esters definitely change as the specimens stand. Of the 58 specimens studied, 25 showed a significant increase in esters or an esterification on standing, ranging from 11 to 74 mg. per 100 c.c.; 9 showed a significant decrease, or a hydrolysis, on standing, ranging from –5.5 to –19 mg. per 100 c.c.; the remaining 24 specimens showed little change.

TABLE II

CHOLESTEROL ESTER (MG./100 C.C.)

(RESULTS OF 20 CASES FROM A SERIES OF 58* CASES)

(WHOLE BLOOD†)

TOTAL CHOLESTEROL	CHOLESTEROL ESTER		
	IMMEDIATE VALUES	24-HOUR VALUES	DIFFERENCE
167	83.5	84	+ 0.5
219	65.5	100	+34.5
128	53	127	+74.0
156	82.5	91.5	+ 9.0
144	61.5	81	+19.5
181	118.5	155	+36.5
203	160	172	+12.0
225	80	106.5	+26.5
154	60.5	60	– 0.5
183	64	101	+37.0
186	126.5	126	– 0.5
214	120.5	169	+48.5
218	126	126	0.0
175	101.5	90	–11.5
233	65.5	66	+ 0.5
169	98	95.5	– 2.5
138	64	64	0.0
155	103.5	107	+ 3.5
163	79	90.5	+11.5
164	92	93.5	+ 1.5

*Complete protocols available on request.

†Similar results were obtained with plasma and with serum.

These results confirm the presence of esterase activity in human bloods.[3] The presence and extent of that activity are unpredictable and varied and, therefore, must be evaluated for each specimen.

Since the percentage of cholesterol esters in the total cholesterol is used clinically as a diagnostic aid in liver damage, the determination, to be of value, must be made on freshly drawn blood.

The original value for cholesterol or cholesterol esters does not indicate the degree or direction of the possible changes on standing.

To establish whether the "enzymatic" activity present in some bloods and absent in others is transitory or is relatively constant, second determinations were made on the same patients a week later. The presence of the activity was shown to be relatively constant.

CONCLUSIONS

1. The total cholesterol content of human blood, plasma, or serum does not change on standing.

2. The value of cholesterol esters in human blood, plasma, or serum may or may not change on standing.

3. Cholesterol determinations on whole blood, plasma, or serum need not be performed immediately after the blood is drawn. Cholesterol ester determinations must be made immediately.

4. The presence of an "esterase activity" producing an hydrolysis of cholesterol esters in some specimens and an esterification of cholesterol in others is confirmed. The nature of this activity, and why it is found in some bloods and not in others, is being investigated.

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THE QUANTITATIVE DETERMINATION OF BLOOD IN HUMAN FECES*

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INTRODUCTION

ALTHOUGH there are many references in medical literature dealing with problems associated with the detection and clinical significance of occult blood in the stomach and intestinal contents of patients suffering from hemorrhage within the gastrointestinal tract, there are very few papers approaching the subject from the standpoint of quantitative measurement. Abrahams[1] and Bramkamp[5] reported experiments designed to ascertain the smallest quantity of blood which after ingestion would produce a stool giving a positive reaction to the benzidine test for occult blood. Later Daniel and Egan,[6] working with larger quantities of blood, found that 50 to 80 c.c. of blood must be ingested by adults on a milk and cream diet in order to produce a so-called "tarry" stool. Although van Eck[7] published a method for the quantitative determination of blood in human feces, careful search of the literature has failed to produce any evidence of its practical application in clinical medicine. In a recent paper[2] Andrews and Brooks outlined a procedure for the quantitative determination of blood in the feces of sheep by means of the Evelyn Photoelectric Colorimeter, incorporating the technique of Bing and Baker[4] for the hemoglobin determination. Since we were of the opinion that one of the more important uses of this method lies in its application to the quantitative study of hemorrhage within the human gastrointestinal tract, we carried out the following experiments.

MATERIALS AND METHODS

The materials and reagents necessary for the work, as outlined in the experiments are as follows:

1. Blood-free human feces.
2. 50 c.c. round-bottomed heavy glass centrifuge tubes.
3. Blood of known hemoglobin content.
4. Accurately calibrated pipettes of 1, 2, 4, 5, 10, 15, and 20 c.c. capacity.
5. Glacial acetic acid.
6. 50 c.c. volumetric flasks.
7. 10 c.c. volumetric flasks.

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8. Purified benzidine reagent of Bing.[3]*
9. Test tubes for the Evelyn photoelectric colorimeter.
10. 3 per cent hydrogen peroxide.
11. Paraffined corks to fit the colorimeter tubes.
12. No. 660 red filter for the colorimeter.

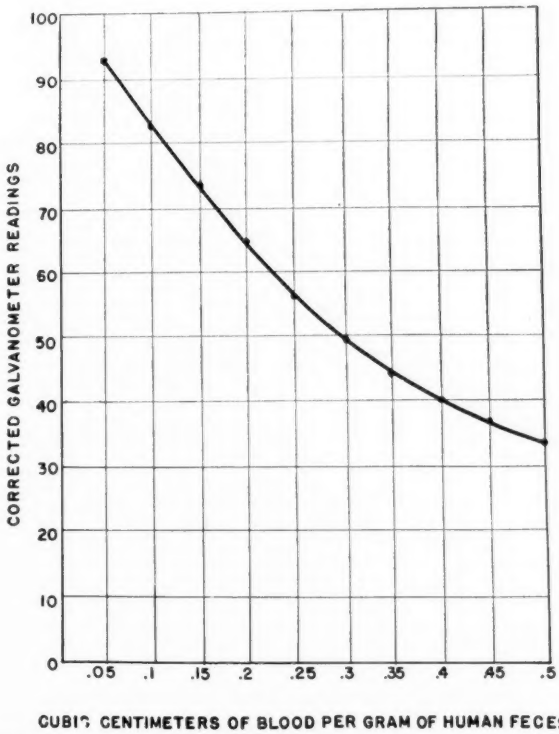

Fig. 1.—Curve showing the corrected galvanometer readings obtained from the analysis of 1 Gm. portions of human feces to which had been added the indicated volumes of calf blood containing 9 Gm. of hemoglobin per 100 c.c.

PROCEDURE

We first placed ourselves on a meat-free diet in order that the feces we passed would not contain ingested blood pigment. To 1 Gm. portions of blood-free human feces collected on the third day, artificial mixtures of known amounts of calf blood, containing 9 Gm. of hemoglobin per 100 c.c. were added, and a graph (Fig. 1) relating the quantity of blood to the colorimetric measurement

*Dissolve 20 Gm. of benzidine base in 200 c.c. of 95 per cent ethyl alcohol with the aid of gentle heat. Filter off insoluble material. Add 1 Gm. of blood charcoal (extracted with hydrochloric acid and reactivated) to the filtrate, stir, and maintain at a temperature of 50° ± 10° C. for fifteen minutes. Filter while warm and wash charcoal with 10 c.c. of warm ethyl alcohol. Repeat treatment with charcoal until filtrate is almost colorless. To filtrate add 135 c.c. of distilled water, or sufficient water to make alcohol 50 to 60 per cent by volume, and warm if necessary to obtain a clear solution. Allow solution to stand in the refrigerator for twenty-four to forty-eight hours to crystallize the benzidine. Filter on a Buchner funnel with suction, wash with cold 50 per cent alcohol, and continue suction until crystals are dry. Preserve in a bottle protected from light.

Prepare the reagent by dissolving with the aid of heat 1 Gm. of purified benzidine in 20 c.c. of glacial acetic acid. Add 30 c.c. of distilled water and 50 c.c. of 95 per cent ethyl alcohol.

Test by placing 2 c.c. of the reagent in a test tube, add 1 c.c. of distilled water and 1 c.c. of 0.6 per cent hydrogen peroxide, and mix. No color, or only a very slight yellow color, should develop in two hours. If the mixture is then diluted to 25 c.c. with 20 per cent acetic acid and viewed through a thickness of about 1 cm., it should be practically indistinguishable from distilled water.

was plotted, as was done in the original paper.[2] The operations necessary for obtaining the data for the curve are as follows:

1. Weigh thirty-two 1 Gm. portions of blood-free human feces on an analytical balance and place each portion in a clean, dry, 50 c.c. centrifuge tube.

2. Make a 1:10 dilution of a blood sample of known hemoglobin content with distilled water and mix thoroughly.

3. Number each centrifuge tube and carefully add the required amounts of diluted blood to each of the 1 Gm. portions of feces, setting up the tubes in triplicate for each point on the curve to be determined. Mix the blood solution thoroughly with the feces, using individual glass stirring rods, and leave the rods in the tubes. Use the remaining two samples as duplicate blanks with blood omitted.

4. Add 10 c.c. of distilled water to the blanks and proportionately less to the remaining tubes, so that the total volume of fluid added will be 10 c.c. Mix thoroughly.

5. Add 15 c.c. of glacial acetic acid to each tube and mix. Heat and remove each tube as soon as the contents begin to boil vigorously. Care must be taken to prevent the contents from boiling over. (The heating was facilitated by putting tubes in wire baskets and placing them on an electric hot plate.)

6. Push particles adhering to the walls of the tubes back into the solution, remove the stirring rods, and arrange them according to number on the edge of a table with the acid-covered ends projecting so that none of the material on the rods may be lost. Centrifuge the tubes at approximately 2,700 r.p.m. for five minutes.

7. Decant the supernatant fluid into properly numbered 50 c.c. volumetric flasks. After transferring the contents of each tube, wash off the decanting rod with a small amount of glacial acetic acid.

8. Make three similar extractions, each with 10 c.c. of glacial acetic acid, and bring the contents of the 50 c.c. volumetric flasks to volume with glacial acetic acid and mix thoroughly.

9. Put 1 c.c. aliquots from the 50 c.c. volumetric flasks into 10 c.c. volumetric flasks, and bring the contents of the 10 c.c. volumetric flasks to volume with glacial acetic acid and mix.

10. Put 2 c.c. of the purified benzidine reagent into the bottom of each colorimeter tube, and add 1 c.c. aliquots from the 10 c.c. volumetric flasks and mix.

11. Add 1 c.c. of a 0.6 per cent hydrogen peroxide solution (3 per cent commercial solution freshly diluted with 4 parts of distilled water) and mix. Close with paraffined corks and allow the tubes to stand for two hours.

12. Dilute with 20 c.c. of a 20 per cent solution of glacial acetic acid in distilled water, insert corks, and invert the tubes several times to mix the contents. Allow to stand for at least eight minutes.

13. Adjust the colorimeter so that the blank reads 100 on the galvanometer scale when a No. 660 red filter is used. With this adjustment make the colorimetric readings on solutions derived from the feces-blood mixtures, and plot the average readings of the triplicates against the volumes of blood employed.

After we had been on a meat-free diet for at least three days, the volumes of blood (indicated in Table I) from the same calf that furnished the specimens for making the reference curve were diluted with 50 c.c. of distilled water and ingested. Each stool was then collected, weighed, thoroughly mixed by means of a spatula, and analyzed quantitatively for blood pigment according to the procedure outlined. Additional blood, however, was not added to the feces, 10 c.c. of distilled water were added to the blank and also to the feces containing blood, and one blank and triplicate samples of the unknown were sufficient for the analysis. The galvanometer readings obtained from the fecal extract containing the unknown quantity of blood were then translated into cubic centimeters of blood by means of the reference curve.

RESULTS

The results of the analysis of the fecal specimens passed subsequent to the ingestion of calf's blood containing 9 Gm. of hemoglobin per 100 c.c. are shown in Table I.

TABLE I

QUANTITY OF INGESTED CALF BLOOD CONTAINING 9 GM. OF HEMOGLOBIN PER 100 C.C. RECOVERED FROM HUMAN FECES

EXPERIMENT NO.	PERSON INGESTING BLOOD; DATE	VOLUME INGESTED	DAY	TIME INGESTED	TIME STOOL PASSED	HOURS AFTER INGESTION	WEIGHT OF STOOL	AVERAGE GALVANOMETER READING	BLOOD INDICATED PER GM. OF FECES	BLOOD RECOVERED	TOTAL BLOOD RECOVERED	
		c.c.		*hour*	*hour*	*number*	*Gm.*		*c.c.*	*c.c.*	*c.c.*	*per cent*
1	J. O. G. May 23, 1941	61	1	3:30 P.M.	5:00 P.M.	1.5	63	93	0.05	3		
			2	----	9:30 A.M.	18.0	971	93	0.05	49		
			3	----	2:00 P.M.	46.5	244	100	0.00	0	52	85
2	J. O. G. June 3, 1941	65	1	2:30 P.M.	----	----	---	--	----	--	--	
			2	----	9:00 A.M.	18.5	154	70	0.17	26		
			3	----	1:30 P.M.	47.0	20	76	0.13	3		
			4	----	9:00 A.M.	66.5	53	89	0.07	4		
			5	----	9:00 A.M.	90.5	100	100	0.00	0	33	51
3	J. S. A. June 3, 1941	65	1	2:30 P.M.	----	----	---	--	----	--		
			2	----	8:30 A.M.	18.0	136	89	0.07	10		
			3	----	9:30 A.M.	43.0	159	80	0.10	16		
			4	----	9:00 A.M.	66.5	95	100	0.00	0	26	40
4	J. S. A. May 23, 1941	61	1	3:30 P.M.	----	----	---	--	----	--		
			2	----	9:00 A.M.	17.5	65	93	0.05	3		
			3	----	9:00 A.M.	41.5	52	72	0.16	8		
			4	----	9:00 A.M.	65.5	93	83	0.10	9		
			5	----	No specimen							
			6	----	8:30 A.M.	110.5	95	100	0.00	0	20	33

The data in Table I show that 33 to 85 per cent of the ingested blood was recovered from the feces. All the specimens containing any appreciable quantity of blood were noticeably darker in color than the blood-free feces, but none of them in our opinion could be called "tarry." In Experiment 1 the blood either acted as a purgative or was accompanied by some substance which had a similar action. The rapid removal of the blood in this instance resulted in the recovery of 85 per cent within eighteen hours after ingestion. The re-

maining experiments show a progressively smaller percentage of recovery, and in general, indicate a negative correlation between the length of time the blood was retained in the digestive tract and the quantity recovered from the feces. Bramkamp[5] reported that pancreatic digestion in vitro reduced the strength of the color reaction between hemoglobin and benzidine by 50 per cent. This prolonged action of digestive juices plus the increased absorption of the blood from the intestine in those instances where the blood was retained in the digestive system for several days would explain the decrease in the apparent quantity of hemoglobin recovered.

A practical test of the method was made on the feces of a patient in the hospital of the School of Tropical Medicine who was suffering from profuse hemorrhage resulting from a condition diagnosed as ulcerative colitis. The results of these tests which were made during the time the patient was hospitalized and placed on a bland ulcer diet with rest in bed are shown in Table II. Because of the large amount of blood pigment in the first two fecal specimens obtained from this patient, four additional extractions with glacial acetic acid were necessary before all the hemoglobin was removed. In this instance the acid extracts were decanted into a 100 c.c. volumetric flask. The blood pigment was so highly concentrated in the extract that it was necessary to use a fraction of a cubic centimeter of the diluted extract in order to obtain a color intensity that could be measured in the colorimeter; hence the multiplying factors shown in column 5.

TABLE II

QUANTITY OF BLOOD RECOVERED FROM THE FECES OF A PATIENT SUFFERING FROM ULCERATIVE COLITIS

DATE	WEIGHT OF STOOL	AVERAGE GALVANOMETER READINGS	BLOOD INDICATED	MULTIPLYING FACTOR	BLOOD* INDICATED IN ONE GM. OF FECES	TOTAL BLOOD* RECOVERED
	Gm.		*c.c.*		*c.c.*	*c.c.*
10/10/40	150	53	0.275	16	4.40	660
10/11/40	No specimen					
10/12/40	107	93	0.050	8	0.40	43
10/13/40	135	89	0.070	0	0.07	9
10/14/40	90	108	0.000	0	0.00	0

*Volume of blood computed on the basis of 9 Gm. of hemoglobin per 100 c.c.

The data in Table II show clearly the severity of the hemorrhage at the beginning of treatment and the gradual cessation of the bleeding under proper care.

DISCUSSION

At first glance the time and technical difficulties involved might make the method outlined appear to be impractical. However, we found that once the reference curve was made, the routine was so well known that tests on specimens containing unknown quantities of blood were easily and quickly carried out. One and one-half hours would probably be a conservative estimate of the time consumed in running a test consisting of one blank and triplicate unknowns. This period does not include the time required for color development.

Five points were emphasized as being important in the successful carrying out of the procedure for the quantitative determination of blood in the feces of sheep. These points are:

1. Use the same lot of purified benzidine reagent for plotting the reference curve and for making the determinations on the unknown in any one experimental series.
2. Always make determinations on fresh fecal specimens.
3. Use meticulous care in weighing, pipetting, and diluting operations.
4. Use fresh glacial acetic acid fecal extracts in making colorimetric comparisons.
5. Do not use blanks for reference points for any other similar sample containing blood unless the two tubes have been diluted at the same time.

In the application of the method to human feces the observance of these measures is just as important as it was in the previous work. The reader is referred to the original paper for further discussion.

SUMMARY AND CONCLUSIONS

A modification of a method for the quantitative determination of blood in the feces of sheep is described for use with human feces. By its use 33 to 85 per cent of ingested blood was recovered from the feces. A negative correlation was observed between the percentage recovered and the length of time the blood remained in the digestive tract. This decrease in the amount of blood recovered was considered to be due to the action of digestive juices and to absorption within the digestive tract.

The method was tested on the feces of a patient bleeding profusely from a condition diagnosed as ulcerative colitis. During the period the patient was under treatment, the tests demonstrated the severity of the hemorrhage at the beginning of treatment and the gradual cessation of bleeding during the days that followed until no more fecal blood was demonstrable.

The results of the tests indicate that the method described can be used for the purpose of estimating the amount of blood passed in the feces of human patients suffering from gastrointestinal hemorrhage.

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THE ADAPTATION OF MARSHALL'S MICRO SULFONAMIDE METHOD TO THE PHOTOELECTRIC COLORIMETER*

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THE increasing importance of the sulfonamides in the treatment of various bacterial infections has resulted in the development and extensive use of several methods for determining these substances in the blood, urine, and body tissues. Of these methods those of Marshall,[6] and Bratton and Marshall,[2] are perhaps the most extensively used in this country. In this report Marshall's improved method[6] has been adapted for use in the determination of sulfanilamide, sulfapyridine, sulfathiazole, sulfadiazine, and sulfaguanidine† with the Evelyn photoelectric colorimeter[4] which is a stabilized direct reading single-photocell type instrument.

The introduction by Bratton and Marshall of N-(1-naphthyl) ethylenediamine dihydrochloride as the coupling reagent to replace the dimethyl alpha-naphthylamine, which had been previously employed, has eliminated some of the objections to the Marshall method advanced by Andrews and Straus.[1] Sunderman and Pepper[7] reported a diminution in recovery of sulfathiazole from whole blood when using the coupling agent (dimethyl-alpha-naphthylamine) of Marshall's original procedure. It is now easily possible to complete a determination by Marshall's method in approximately fifteen minutes, and the color produced remains stable for a period of two or more hours. The method recently proposed by Churg and Lehr,[3] while equally rapid for the free sulfonamide, is somewhat slower for the total determination than the procedure presented here.

The method to be described has proved rapid, simple, and accurate, as evidenced by high percentage recoveries of the various sulfonamides which have been added to whole blood (dog and human), and may be used with either 0.2 or 0.1 c.c. of blood. Certain previous objections or uncertainties of the method, such as the length of time required for analysis, instability of color, or incomplete recoveries, are eliminated by the use of Marshall's more recent coupling agent N-(1-naphthyl) ethylenediamine dihydrochloride and by the use of the photoelectric colorimeter.

Reagents.—1. A solution of trichloroacetic acid containing 15 Gm. dissolved in water and diluted to 100 c.c.

2. A 0.1 per cent aqueous solution of sodium nitrite—this reagent should preferably be made up at weekly intervals.

*From the Aero Medical Unit, Army Air Forces, Materiel Center, Wright Field, Dayton.

†Supplies of these sulfonamides were obtained from Merck & Co., Inc., and the Lederle Laboratories, Inc.

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3. An aqueous solution of N-(1-naphthyl) ethylenediamine dihydrochloride containing 100 mg. per 100 c.c. This solution should be kept in a dark-colored bottle.

4. 4 N hydrochloric acid.

5. A solution containing 0.5 Gm. of ammonium sulfamate per 100 c.c. This solution should be freshly prepared each week and discarded at the first sign of contamination with molds.

6. Stock solutions of the various sulfonamides in water containing 200 mg. per liter. From this solution a series of dilutions were prepared for use in determining the respective calibration curves.

PROCEDURE

Blood for 0.2 c.c. Samples.—Two-tenths cubic centimeter of blood (oxalated or heparinized) is delivered into a colorimeter tube* containing 7.8 c.c. of distilled water. Proteins are precipitated by adding 2.0 c.c. of trichloroacetic acid, and removed by filtering. (Whatman No. 12 has proved suitable.)

Blood for 0.1 c.c. Samples.—One-tenth cubic centimeter of blood is added to 3.9 c.c. of distilled water in a graduated centrifuge tube and 1.0 c.c. of trichloroacetic acid is added for protein precipitation. Centrifuge for four to five minutes. Two cubic centimeter samples of supernatant fluid are then treated as outlined for the free and total sulfonamide determination.

Free Sulfonamide.—Two cubic centimeter samples of the blood filtrate are delivered into a colorimeter tube. One-half cubic centimeter of sodium nitrite is added. After two minutes 0.5 c.c. of the ammonium sulfamate is added. After another two minutes, 0.5 c.c. of N-(1-naphthyl) ethylenediamine dihydrochloride is added. The solution is then diluted to 10 c.c. by adding 6.5 c.c. of distilled water, and the color density is determined in the photoelectric colorimeter with a green filter (Corning) No. 540.

Total Sulfonamide.—After the sample for determination of the free sulfonamide is removed, the remainder of the blood filtrate is placed in a graduated centrifuge tube, the volume is observed and 0.4 c.c. of 4N HCl is added. It is heated in boiling water for twenty minutes, cooled, and adjusted to original volume with distilled water. This dilution precaution must be observed to avoid considerable error. Subsequent procedure is identical to that outlined above for free determination.

Calibration Curves.—Dilutions of a stock solution (200 mg. per liter) were prepared in concentrations ranging from 0.5 to 15.0 mg. per 100 c.c. and containing 18 c.c. of trichloroacetic acid per 100 c.c. A 0.2 c.c. sample of these respective stock dilutions was added to 8.1 c.c. of distilled water to which 0.2 c.c. of trichloroacetic acid has been added. One-half cubic centimeter of each reagent was then added at two-minute intervals. The calibration curves of the respective sulfonamides were then determined from the colorimeter readings of samples prepared in duplicate as above.

*These tubes are of especially selected glass and are furnished by the Rubicon Company, Philadelphia, Pa. from whom the Evelyn photoelectric colorimeter and filters may also be obtained.

Reagent Blank.—A stock blank is prepared by adding 2.0 c.c. of trichloroacetic acid to 8.0 c.c. of distilled water. A 2.0 c.c. sample is then delivered into a colorimeter tube, and 0.5 c.c. of each of the reagents is added at two-minute intervals. Six and one-half cubic centimeters of distilled water are added, the tube is placed in a colorimeter and the galvanometer is adjusted to 100, the reagent blank thus serving as the 0.0 mg. per 100 c.c. standard.

RESULTS

Calibration curves relating concentration (milligrams) to photoelectric density (2 – log G) of these sulfonamides were found to be linear from a concentration of 0.5 mg. per 100 c.c. to 10 mg. per 100 c.c. for all the sulfonamides tested. Concentrations in excess of 10.0 mg. per 100 c.c. deviate somewhat from a straight line, particularly in the case of sulfanilamide. The calibration factors (K) derived from the respective curves were used in the calculation of the per cent recoveries of the various sulfonamides from whole blood. These factors were 50.6 for sulfanilamide, 33.5 for sulfapyridine, 36.0 for sulfathiazole, 36.9 for sulfadiazine, and 39.3 for sulfaguanidine. Standard deviations of these factors were 1.2, 1.2, 0.2, 0.2, and 0.2, respectively.

The percentage error for the 0.2 c.c. procedure in the recoveries from whole blood concentrations of 10 mg. per 100 c.c. varied from 0.2 to 2 per cent, and from 1 to 2 per cent in recoveries from concentrations of 1 mg. per 100 c.c. This probably represents the limit of accuracy for the 0.2 c.c. method as described here. The accuracy of the 0.1 c.c. procedure is somewhat less.

Results reported here were calculated by use of the formula:

$$\text{Mg. per 100 c.c.} = \frac{(2 - \log G)}{K} \times \frac{\text{dilution used}}{\text{c.c. of filtrate used}} \times 100$$

G is the galvanometer reading and *K* the calibration factor for the respective sulfonamide. For a 1:50 blood dilution the use of 2 c.c. blood filtrates and the twenty-minute hydrolysis period have been found to give satisfactory results. Color developed in the blood blank is negligible, since in the 1:50 dilution of blood the correction due to this was found to be about 0.08 mg. per 100 c.c. The reagent blank shows no measurable color development subsequent to its exposure to ordinary indoor room lighting conditions over a period of three to four hours. If analyses continue beyond this period of time, a fresh blank should be prepared if precise results are desired. The color developed as a result of the coupling reaction is stable for a period of two or three hours, after which its density slowly alters, and readings made after longer periods of time may be considerably in error.

DISCUSSION

The use of calibration curves for the respective sulfonamides seems desirable, since they are linear over the range of 0.5 to 10 mg. per 100 c.c. and quite reproducible. Their use avoids any uncertainty that arises from the employment of a standard whose color density, as mentioned above, is slowly altering. The green filter recommended (Corning No. 540) may be used for all the sulfonamides reported, since the wave length of the maximum absorption for

the respective compounds has been found by Feinstone and associates[5] to be practically identical. As pointed out by Bratton and Marshall[2] the method is extremely sensitive, and concentrations of 1 μg can be easily detected.

Diazotization with the nitrite and destruction of the excess nitrite by the ammonium sulfamate are rapid reactions, both being complete within thirty to sixty seconds. Formation of the azo dye by the coupling reaction is slower and the two-minute interval recommended must be observed for reproducible results. Complete analyses for the free drug require twelve to fifteen minutes, while the total sulfonamide determination may be completed in thirty-five to forty minutes.

N-(1-naphthyl) ethylenediamine dihydrochloride as the coupling agent possesses several distinct advantages, some of which have previously been pointed out by Bratton and Marshall. One of these is its speed, the method reported here being equally as rapid as that suggested by Andrews and Straus,[1] or Churg and Lehr.[3] The procedure proposed by the latter workers requires also the undesirable use of an empirical factor to correct for a decrease in the color intensity due to the additions of hydrochloric acid in the total sulfonamide determination. No difficulty in securing satisfactory recoveries of sulfathiazole added to whole blood has been experienced. The necessity, therefore, for centrifuging the protein precipitate as Sunderman and Pepper[7] reported, has been removed.

It is important in all procedures to observe the time interval between the addition of the various reagents and also to agitate slightly the solution during the various steps. Unless these precautions are observed in the preparation of blanks or samples, turbidities may result, or improper unstable colors may develop.

SUMMARY

1. An adaptation of Bratton and Marshall's method for the determination of sulfonamides in 0.2 and 0.1 c.c. blood samples is described for use with the photoelectric colorimeter.

2. Calibration curves obtained with the Evelyn photoelectric colorimeter over a 0.5 to 15 mg. per 100 c.c. range of concentration has been determined for sulfanilamide, sulfapyridine, sulfathiazole, sulfadiazine, and sulfaguanidine. The calibration factors (K) and standard deviations of these curves are presented.

3. The per cent error in the recoveries of these various sulfonamides as determined in concentrations of 1 and 10 mg. per 100 c.c. varied from 0.2 to 2.0 per cent.

4. The method as presented here is simple, sensitive, relatively rapid, and gives precise reproducible results over a range of concentrations varying from 0.5 to 15.0 mg. per 100 c.c.

I wish to express my appreciation to Dr. J. W. Heim for helpful criticism and suggestion.

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A CLINICAL MICROMETHOD FOR THE DETERMINATION OF BLOOD UREA*

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THE diagnostician realizes that functional disturbances may be the first indication of anatomic changes. A deviation from normal nonprotein nitrogen of the blood is often a sign of a metabolic disturbance, if not an indication of a change to a definite pathologic stage. The study of the total nonprotein nitrogen or of urea nitrogen in the blood, although of diagnostic value in a number of disturbances, is most important when confronted with the problems of renal dysfunction.

The determination of the total nonprotein nitrogen of the blood, while most frequently employed, has several disadvantages. The method requires a well-equipped laboratory and a technician with good training. The nonprotein nitrogen fraction of the blood contains about 25 per cent of undetermined or rest nitrogen, made up of nitrogenous substances of practically unknown metabolic significance, especially under pathologic conditions. The relative increase in urea nitrogen is greater than the nonprotein nitrogen rise when dealing with certain renal disturbances. It must be kept in mind that urea is largely a waste product, whereas the undetermined nitrogen is anabolic. We believe, therefore, that the variations in urea alone serve as a better diagnostic guide than the nonprotein nitrogen.

A recently published procedure[1] for the determination of urea nitrogen has been modified to give the physician a method which does not require a laboratory, and at the same time is accurate enough to satisfy his diagnostic needs.

The enzyme urease converts urea into ammonium carbonate. The addition of the alkali sodium carbonate liberates ammonia, which is absorbed in the present method through "passive distillation" in hydrochloric acid.

The apparatus consists of a cylindrical vessel and a small glass cup fused to a rod. The cup and rod are attached to a cork which is inserted into the cylinder (Fig. 1).

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The procedure is as follows: 0.1 ml. of blood from the ear lobe or finger tip is measured into the cup. One urease tablet, containing both urease and the necessary buffer salts, is crushed, and one-third to one-half of this powder is added to the blood contained in the cup. Blood and urease are mixed by gentle agitation, care being taken to avoid any liquid spilling over the rim of the cup. The stopper with the cup is inserted into the vessel and is allowed to stand for about one hour at room temperature. The cup assembly is then

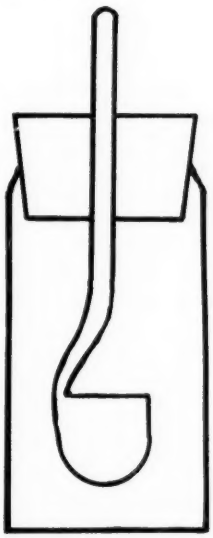

Fig. 1.

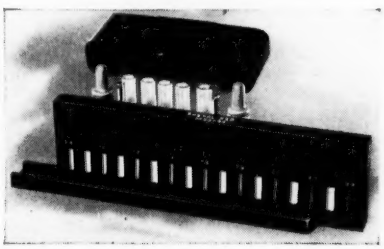

Fig. 2.

removed, and 1 ml. of approximately tenth-normal hydrochloric acid is carefully measured into the cylinder. Three drops of a concentrated solution of sodium carbonate are added to the blood in the cup, and the stopper is *quickly* inserted, to avoid any possible loss of ammonia. The vessel is gently agitated by a rotary motion, to mix the contents of the cup. The apparatus is allowed to stand (at room temperature) for about eight hours, or still better overnight. The stopper with the cup is then removed, 3 ml. of water are added to the fluid in the cylinder and the contents are mixed by rotation. One milliliter of Nessler reagent (Bock-Benedict modification[1]) is added, the solutions are mixed again and allowed to stand for eight to ten minutes. This is necessary for full development of the color. The liquid is transferred to a small tube, which in

turn is inserted into the holder of a Taylor* slide comparator (Fig. 2). The movable slide of the instrument has a set of tubes containing permanent standards, the colored tubes being interspersed by tubes containing water. The slide is moved back and forth until the unknown is flanked by those two standard tubes which match the closest. The standards are permanent, consisting of a mixture of ferric and cobaltous chloride in acid solution and arranged to give a direct reading in milligrams per 100 c.c. of blood, ranging from 10 mg. to 100 mg. of nitrogen.

The color standards are made as follows:

$FeCl_3 \cdot 6H_2O$: 100 Gm. in 1,000 c.c., using 1 per cent (by weight) hydrochloric acid as solvent.

$CoCl_2 \cdot 6H_2O$: 100 Gm. in 1,000 c.c., using 1 per cent (by weight) hydrochloric acid as solvent.

UREA NITROGEN (MG. PER 100 C.C.)	$FeCl_3$ SOLUTION (C.C.)	$CoCl_2$ SOLUTION (C.C.)	
10	11.30	4.35	
20	20.45	9.45	
30	29.50	13.70	Make total volume to 100 c.c., using 1 per cent (by weight) hydrochloric acid solution
40	37.05	15.55	
50	50.30	21.55	
60	52.70	26.35	
70	54.35	31.15	
80	56.00	36.00	
100	54.55	45.50	

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*The complete equipment may be obtained from W. A. Taylor and Company, 7300 York Road, Baltimore, Md.

DEPARTMENT OF ABSTRACTS AND REVIEWS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

ALCOHOL, The Perchlorate Method for Determining Concentration of, in Expired Air, Jetter, W. W., and Forrester, G. C. Arch. Path. 32: 829, 1941.

A critical evaluation of the perchlorate method for the determination of breath alcohol has been made, and the following data have been presented: When the test is properly made, there is no loss of alcohol or of carbon dioxide before the breath reaches the absorption tubes; reducing substances in the breath other than alcohol are negligible in their effect; the presence of acidosis or alkalosis in the test subject materially affects results, but these conditions may be controlled or eliminated by proper precautionary measures.

In a series of 79 cases in which breath and direct blood analyses were made, the deviation did not exceed 16 per cent in any instance.

In view of the simplicity of operation and the ease with which the instrument can be transported, this method is well adapted for use by law enforcement agencies.

AMYLOIDOSIS, Standardization of the Congo Red Test for, Taran, A., and Eckstein, A. Am. J. M. Sc. 203: 246, 1942.

A standardized method for the Congo red test for amyloidosis is presented.

The dye is administered according to body weight. It was found that 1 c.c. of a 1 per cent aqueous solution of the dye per 10 pounds of body weight yielded more accurate tests than the fixed amount customarily used.

Tests were performed on amyloid and nonamyloid patients, using both the usual technique of injecting 10 c.c. of dye regardless of body weight and the standardized method.

In one nonamyloid group patients were selected whose body weights were nearly the same (range between 95 and 105 pounds) and in the other those whose body weights varied considerably (range between 50 and 175 pounds). The former group showed consistently reliable four-minute specimens, while the latter gave four-minute specimens which differed as much as 75 per cent. In some cases the per cent absorption could not be computed because of insufficient dye in the four-minute specimens.

In the amyloid group 90 to 100 per cent absorption was a consistent finding. This is in so close correlation with previous work that such results must be obtained to indicate amyloidosis. In all but 11 cases the four-minute specimens contained sufficient dye to be considered as appropriate standards.

These 11 cases were investigated further along with 24 of the other amyloid cases, and it was found expedient to draw a two-minute specimen in addition to the four-minute and one-hour specimens. A group of 60 nonamyloid cases was chosen as controls. In all of the amyloid cases, the two-minute specimens showed good color intensity and the differences in color between them and the four-minute specimens were marked (as high as 75 per cent). There were only slight differences in color intensity between the two-minute and four-minute specimens in the nonamyloid group, the highest per cent variation being 15.

The two-minute specimen was adopted as the colorimetric standard in place of the four-minute specimen.

Since the inception of this study 21 of the 42 amyloid patients have come to autopsy and each revealed amyloidosis of greater or lesser degree. Twenty of the nonamyloid patients have come to autopsy and only one case revealed a moderate degree of amyloidosis.

The standardized method has improved the accuracy and reliability of the test as a diagnostic aid.

The authors' method follows:

Inject 1 c.c. of a 1 per cent aqueous solution of Congo red per 10 pounds of body weight intravenously. At the end of exactly four minutes, and then one hour, after the injection, obtain blood specimens of 10 c.c. each and place them into clean, dry test tubes. Allow the bloods to clot and retract and then centrifuge them at a moderate speed for ten minutes. Aspirate off the clear sera and place them into graduated centrifuge tubes. Add acetone equal to the volume of serum in each tube and shake them well. Centrifuge tubes for ten minutes at a moderate speed. Pour off the supernatant fluids and place them into micro cups of a colorimeter to be compared. Set the four-minute specimen at 20 mm. and read the one-hour specimen.

Calculation:

$$100 - \frac{\text{Reading of four-minute specimen}}{\text{Reading of one-hour specimen}} \times 100 = \text{Per cent absorption.}$$

For example: The four-minute specimen is set at 20 mm. in the colorimeter. The one-hour specimen reads 25. Therefore, the per cent absorption of the dye is 20.

COAGULATION, Blood, Simple Method of Timing, Knauer, C. H. J. Med. Soc. New Jersey **39**: 75, 1942.

An ordinary hypodermic syringe with a slightly larger-sized needle than ordinary is used. If necessary, the small diameter needle will serve. Blood is withdrawn from the median basilic or some other prominent vein. A few bubbles of air are drawn into syringe, and it is allowed to rest on its side for three minutes, after which time it is tilted slowly from end to end so as to permit the bubbles with their interspaces of blood to travel slowly from one end of the barrel to the other at one-quarter minute intervals. It is soon found that the rapidity with which these bubbles travel the length of the syringe becomes diminished with the formation of the clot and that one-half to three-quarters minute transpires between the onset of the clot formation and the actual completion of the process which is noted by the almost immobility of the bubbles.

RHEUMATIC FEVER, Formol-Gel Test in, Butterworth, J. S., and Poindexter, C. A. Am. J. M. Sc. **203**: 278, 1942.

The formol-gel test is essentially a test for hyperglobulinemia and has no correlation with the erythrocyte sedimentation rate.

It is of little or no value in the diagnosis of acute rheumatic fever with or without carditis.

HYPOPROTHROMBINEMIA in Pernicious Anemia, Warner, E. D., and Owen, C. A. Am. J. M. Sc. **203**: 187, 1942.

Patients with Addisonian pernicious anemia in relapse usually show considerable decrease in plasma prothrombin. In the majority of such cases the prothrombin level is found to be between 40 per cent and 65 per cent of the normal. The hypoprothrombinemia is not rectified by large doses of vitamin K. When specific liver therapy is instituted, the plasma prothrombin level promptly shows a marked rise.

PROTHROMBIN DEFICIENCY, Effect of Vitamin K Therapy on the Plasma Prothrombin Level of Patients With, Rhoads, J. E., and Norris, R. F. Bull. Ayer Clin. Lab., Pennsylvania Hosp. **3**: 359, 1941.

The administration of an excess of 2 methyl-1,4-naphthoquinone to surgical patients without hypoprothrombinemia resulted in a slight shortening of the prothrombin time, as determined by the Quick method and a modification thereof.

This difference is not regarded as significant in the number of cases studied, in view of the variations observed among the controls; it is less than the difference between normal controls and the plasma of parturient mothers, observed by Norris and Rush, and much less than was observed in some of their patients.

It is also of interest that six days of therapy yielded no better result than did two days of therapy.

The results indicate, therefore, that the supply of vitamin K is not an important limiting factor in determining the prothrombin level under normal conditions.

There is nothing in the results of the study to suggest that the administration of vitamin K would be valuable in the prevention of hemorrhage in patients who do not have hypoprothrombinemia, nor that it would be dangerous to give the vitamin in considerable excess to patients whose prothrombin levels were already normal.

The method of study is not sufficiently accurate to establish satisfactorily or to exclude a mass action effect of vitamin K on the production of prothrombin.

PROTHROMBIN, Comparison of Bilirubin Values and, in the Umbilical Artery and Vein at Delivery, Norris, R. F., and Bennett, M. C. Bull. Ayer Clin. Lab., Pennsylvania Hosp. 3: 353, 1941.

The average bilirubin content of fetal blood exceeds that of maternal blood at delivery. In individual infants there is a marked variation in the degree of hyperbilirubinemia, which is independent of the amount of bilirubinemia in their respective mothers.

There is no evidence that bilirubin diffuses in either direction through the placenta.

No relationship is demonstrated between the amount of serum bilirubin and the plasma prothrombin in individual infants.

SHOCK, The Vascular and Cellular Dynamics of, Moon, V. H. Am. J. M. Sc. 203: 1, 1942.

An analysis of the sources for disagreement concerning the dynamics of shock indicates that four major causes have operated to confuse interpretations of its phenomena and of their interrelationships.

The first of these was deficient knowledge of capillary reactions and of circulatory disturbances originating in them. A comprehension of these has contributed immeasurably to an understanding of shock resulting from trauma, burns, and other types of tissue damage. It clarifies also the disturbances of fluid balance and of chemical concentrations which are associated regularly with this condition.

Failure to distinguish between shock and hemorrhage has caused much confusion. Hemorrhage, when present, is a potent contributory factor, but the effects of uncomplicated hemorrhages present numerous features opposite in character from those which constitute the syndrome of shock.

Variations in blood pressure used as a criterion, and failure to consider the depressor effects of both anesthesia and losses of blood, have led to undependable conclusions concerning the effects of absorption. Varied forms of experimentation have shown that products derived from normal tissues, independent of narcosis and hemorrhage, will produce the syndrome of shock accompanied by its characteristic physiologic and morphologic features.

Hemoconcentration during life, and the presence of engorged capillaries, stasis, petechiae, and edema after death, indicate endothelial damage as a major factor. The occurrence of these conditions in extensive visceral areas remote from the injury indicates systemic, not local, endothelial damage. Shock, like other conditions of disease, is accompanied by a pattern of morphologic changes which are related etiologically to its mechanism of origin and which corroborate the interpretation of shock as due to endothelial damage.

The major causes for disagreement concerning shock have been discussed. In view of the character and magnitude of these, it is not strange that final clarification of the problem has been delayed.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Lymphatics, Lymph, and Lymphoid Tissue*

A COMPREHENSIVE authoritative volume, this book covers the origin, nature, and function of the elements indicated in the title.

As the writers bring out, study of the physiology of lymph carries us nearer to a study of the life processes of the tissue cells. Lymph circulates from the blood vessels into the tissue spaces surrounding the cells and out again through the lymphatics into the blood vessels. It is this fluid that is of utmost importance in the maintenance of vital cellular equilibrium. Considerable space is necessarily given to the phenomena of capillary permeability, and there is an associated discussion of water balance and electrolyte metabolism. Clinical implications are adequately discussed.

This volume should be of interest to physiologists and, on the clinical side, especially to surgeons, internists, allergists, and dermatologists.

Photomicrography†

A HANDBOOK which adequately covers the entire field of photomicrography, the first portion of *Photomicrography* deals with the physics of photography, especially as applied to the use of the microscope with photographic cameras. The major portion of the book deals with equipment and its use. This extends from simple homemade equipment, through the use of candid camera attachments, such as the Leica and Contax, up to complicated apparatus of highest precision, including the electron microscope. Fifty pages are given to darkroom technique, developing and printing, and there is an abundance of formulas for developing solutions. In this way the book is complete, requiring little or no reference to other volumes. It is abundantly and very well illustrated.

Biological Symposia‡

THIS volume is devoted entirely to the structure and experimental physiology of muscle. It contains sixteen essays by various authors. One is likely to find the answer to whatever one would wish to know concerning muscle physiology, somewhere in this volume. Unfortunately there is no index. Also, the binding does not indicate the subject matter. Although the book is devoted to one subject, muscle, one must look inside to discover this.

Subjects discussed include muscle excitability, action, potentials and conduction, activity around the neuromuscular junction, regulation of energy exchange, changes during muscle contraction, the significance of oxidation, efferent innervation, electrolyte equilibrium in muscle, and electric potential changes accompanying neuromuscular transmission. Both smooth and striated muscle are covered.

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*Lymphatics, Lymph, and Lymphoid Tissue. Their Physiological and Clinical Significance (Harvard University Monograph in Medicine and Public Health, No. 2). By Cecil Kent Drinker, M.D., D.Sc., Professor of Physiology, School of Public Health, Harvard University; and Joseph Mendel Yoffey, M.Sc., M.D., F.R.C.S. (Eng.), Senior Lecturer in Anatomy, University College of South Wales and Monmouthshire, Cardiff, Wales. Cloth, 406 pages, $4.00. Harvard University Press, Cambridge, Mass., 1941.

†Photomicrography. By R. M. Allen. Cloth, 365 pages, $5.50. D. Van Nostrand Company, New York, 1941.

‡Biological Symposia—A Series of Volumes Devoted to Current Symposia in the Field of Biology. Edited by Jaques Cattell, Editor of The American Naturalist and American Men of Science. Volume III. Muscle. Cloth, 370 pages. The Jaques Cattell Press, Lancaster, Pa., 1941.

A Manual of Neurohistologic Technique*

THIS small volume presents methods of preparation and staining of nerve tissue for microscopic study. In it the author has collected the best techniques from scattered places in the literature and gives his own critical experiences with them. This should make a handy reference volume for pathologists and neuroanatomists.

An Introduction to Dermatology†

PRIMARILY a condensation of the larger book *Diseases of the Skin* by the same authors, the fourth edition of the book is based on the tenth edition of the larger volume. Even the introduction is large enough, covering 900 pages. It is abundantly and well illustrated, and constitutes an adequate dermatologic reference book for all who are not primarily dermatologists.

Chemical Solutions‡

IN THIS book the author has collected in one place the methods for the preparation of the solutions most commonly used in the average chemical laboratory, such as organic, biochemistry, food chemistry, metallurgy and metallography, bacteriology, gas and fuel analysis, general testing, and water analysis. The solutions are listed alphabetically according to their most common name; when a reagent is known by several names, these names are also included in their proper place in the alphabetical tabulation with proper cross references. Additional valuable information is also given, such as, (a) the uses of each solution; (b) the procedure for use where this is practicable; (c) interfering substances; (d) sensitivity of the test reagent; and (e) general keeping qualities. One or more reference is included for each solution, wherever advisable. A valuable feature is an index in which the various reagents are classified according to their use. All descriptions are concise and to the point, and unnecessary detail is eliminated. It is the opinion of the reviewers that this book should prove a most valuable adjunct to any chemical library.

Chromatographic Adsorption Analysis§

A GOOD technique sometimes renders more service to science than the elaboration of highly theoretical speculations.'' With this introduction from the writings of Claude Bernard, Dr. Strain in this book, Volume II of the series of monographs on Chemical Analysis by the same publishers, has compiled in a single volume practical information on chromatography from widely scattered sources. The remarkable influence of this technique on the progress of many chemical investigations of varied nature, particularly in the field of biological compounds, makes such a book highly desirable. The technique of separation of many types of compounds, organic and inorganic, colorless as well as colored, by adsorption on Tswett columns is described. Many diagrams of apparatus are presented. Various absorbents, their applications, limitations, availability, and choice for certain types of compounds, as well as various methods for filling the columns, are discussed. Selection of solvents and eluents and the many factors influencing their usefulness are presented in detail. The usefulness of the volume is greatly enhanced by the inclusion of an extensive bibliography, with titles from which one can readily find literature pertaining to particular problems, as well as a very good author and subject index which indexes bibliography as well as text. It is a useful

*A Manual of Neurohistologic Technique. By Oscar A. Turner, M.D., New Haven, Conn. Cloth, 73 pages. The C. V. Mosby Company, St. Louis, Mo., 1940.

†An Introduction to Dermatology. By Richard L. Sutton, M.D., Sc.D., LL.D., F.R.S. (Edin.), Emeritus Professor of Dermatology, University of Kansas School of Medicine; and Richard L. Sutton, Jr., A.M., M.D., L.R.C.P. (Edin.), Assistant Professor of Dermatology, University of Kansas School of Medicine. Cloth, ed. 4, 904 pages, with 723 illustrations. The C. V. Mosby Company, St. Louis, Mo., 1941.

‡Chemical Solutions. By Frank Welcher, Assistant Professor of Chemistry. 404 pages, $4.75, D. Van Nostrand Company, Inc., New York, N. Y., 1942.

§Chromatographic Adsorption Analysis. By Harold H. Strain, Ph.D., Carnegie Institution of Washington, Stanford University, Calif. Cloth, 222 pages, 37 illustrations, colored frontispiece. $3.75. Interscience Publishers, Inc., New York, N. Y., 1942.

practical book for the chemical investigator, and its readable style also makes it valuable for others who would like to know more about the technique involved in chromatographic adsorption analysis.

Analytical Chemistry of Industrial Poisons, Hazards and Solvents*

THIS is an exhaustive text dealing with the detection of substances important from the standpoint of industrial health. Thorough discussion is made of air sampling, measurement of gas volume and quantity, absorbers and absorbents, the chemical and microscopic estimation of dust. Then follows specific and detailed description of the detection of silica, the dangerous metals, poisonous compounds of sulfur, and the halogens. The gases, carbon monoxide, carbon dioxide, hydrocyanic, and cyanogen, are subsequently taken up and then a consideration of the various solvents, including the alcohols and glycols. The author closes his text on the very timely topic of the chemical warfare agents. Brief mention is made in each instance on the toxicity and physiologic activity of the materials under consideration. This is an outstanding text which will prove most beneficial to all interested in industrial health. It is most heartily recommended.

H. B. HAAG.

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*The Analytical Chemistry of Industrial Poisons, Hazards and Solvents. By M. B. Jacobs, Food, Drug and Insect Administration, United States Department of Agriculture, 1927; Chemist, Department of Health New York City, 1928; formerly Lt. U. S. Chemical Warfare Service Reserve. 110 illustrations, 661 pages. Interscience Publishers, Inc., New York, 1941.